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AUSTRALASIAN ANNALS OF MEDICINE

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AUSTRALASIAN ANNALS OF MEDICINE

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GLOMERULONEPHRITIS IN CHILDREN : A CORRELATION OF SERIAL PAPER ELECTROPHORESIS WITH THE CLINICAL CONDITION[1]

MAGDA REICH, D. A. COATS AND I. R. MCDONALD
From the Department of Physiology, University of Melbourne

IN acute and subacute glomerulonephritis there is little published work to indicate any progression of the serum protein changes, or any correlation with the clinical course of the diseases. Published electrophoretic patterns in these conditions do not indicate any significant changes in the various protein fractions. This is at variance with our own findings presently to be put forward, and the only possible interpretation seems to be that sporadic examinations have been made either before or after the period in the diseases when we have observed significant and characteristic changes. For this reason we have, over the past two years, made serum electrophoretic studies throughout the course of their disease and in their recovery periods on 15 children suffering from acute and subacute glomerulonephritis. Our interest in this problem arose initially from the observation that anuric patients have a very rapid erythrocyte sedimentation rate. This suggested an alteration in the protein fractions in anuric serum. Electrophoretic studies were therefore undertaken in children during the course of acute glomerulonephritis with anuria and anuria following sulphonamide therapy, and later the investigation was extended to include cases of acute and subacute glomerulonephritis without anuria.

METHOD

The method of Flynn and de Mayo (1951) was used in all estimations with certain modifications.

Whatman number 2 filter paper was used cut into strips 3·5 by 34 centimetres. The buffer solution contained 10·3 grammes of sodium diethyl barbiturate *plus* 1·4 grammes of diethyl barbituric acid made up to one litre in distilled water. This was then adjusted to pH 8·6 having an ionic strength of 0·05M. The current was a direct current of 250 volts and the milliamperage was 3·5.

PROCEDURE

The electrophoretic run was done in a cold room at 5° C.

Buffer was poured into the outer compartments of the tank to equal levels, and distilled water into the middle compartment. The apparatus was then covered and left to equilibriate for several hours.

The filter paper strips were prepared and a faint pencil line was drawn across the middle of each one. Each strip was then soaked with buffer and excess moisture was removed by blotting with a clean towel. The damp strips were then placed horizontally on the tank and 0·03 millilitre of serum was applied. A fine-pointed pipette was used and moved over the mark evenly four or five times, without extension to the edge of the strip. After the serum was absorbed the strips were suspended from the glass rod and placed in the compartments, forming an inverted V. The ends were made to adhere to the walls of the individual compartments. The glass cover was mounted and a direct current as described was passed across each paper. Six electrophoretic separations were done simultaneously and complete separation took place within sixteen to seventeen hours.

[1] This work was supported by a grant from the National Health and Medical Research Council. Received on July 27, 1955.

After this time the moist ends of the paper were blotted and the strips were removed and dried in a horizontal position in an oven at 100° C. for thirty minutes. The strips were then rolled into loose bundles, placed in a beaker and stained as follows:

(*a*) Five minutes in 1% bromophenol blue in saturated alcoholic mercuric chloride ($HgCl_2$) solution. (*b*) Ten minutes in 1% $HgCl_2$ solution in methanol. (*c*) Ten minutes in 1% $HgCl_2$ solution in ethanol. (*d*) Five to ten minutes in methanol. This methanol washing was repeated two or three times until all dye was washed out from the protein-free areas of the paper.

In the cases of M.H. and C.J. the papers were cut into strips, eluted with alkaline methanol and read by direct photometry (Tiselius, Cremer and Deidrich, 1949–1950; and Flynn and de Mayo, 1951). In all other cases the protein-bound dye was evaluated by means of an "EEL" scanner. It was found unnecessary to render the papers translucent for evaluation of the protein-bound dye. When they were made translucent with paraffin oil (Grassmann and Hannig, 1950) the values obtained were somewhat lower, but the relative values of the various fractions one to another were unaltered. This confirms the findings of Sommerfelt (1952).

TABLE I

Essential Features of Case Histories of Children with Acute Glomerulonephritis and Associated Anuria

Initials	Age	Sex	Onset of Preceding Infection (Days Before Admission)	Onset of Acute Nephritis (Days Before Admission)	Date of Admission	Date of Discharge or Death	Maximum Blood Pressure, Systolic and Diastolic	Œdema	Proteinuria	Oliguria or Anuria (from Onset of Acute Nephritis)	Progress
M.H.	9	F.	17	5	17. 6.53	4. 7.53 Died	142/98	+	+	1st to 14th day (15–30 ml. per day)	Diuresis commenced on 26.6.53. Convulsions occurred on 24.6.53, followed by progressive drowsiness and coma till death. Acute nephritis confirmed *post mortem*
C.J. ..	5	M.	—	6	23. 5.53	1. 9.53	140/90	+	+	2nd to 11th day (0–30 ml. per day)	Diuresis commenced on 28.5.53 and condition gradually improved. Discharged with minimal proteinuria and hæmaturia and was well ten months later
B.A.M.	8	M.	14	7	21. 8.54	17.11.54	130/90	+	+++	5th to 17th day (0–60 ml. per day)	Diuresis commenced on 31.8.54. Clinical improvement was slow, and his urine was still of low specific gravity and showed proteinuria and hæmoglobinuria three months after onset of the illness. Two months later he was clinically well and had minimal proteinuria
F.B. ..	10	M.	14	1	10. 7.54	8. 8.54	115/70	++	+	1st to 4th day (no urine)	Diuresis commenced on 10.7.54. Œdema cleared five days and proteinuria 21 days after admission to hospital. Discharged with minimal hæmaturia and was well five months later.
P.B. ..	7	F.	14	4	23. 8.53	21. 9.53	120/70	-	+	1st to 10th day (no urine)	Diuresis commenced 29.8.53 and condition gradually improved. Discharged clinically well with minimal proteinuria and hæmaturia
B.K.M.	7	M.	10	8	6. 5.53	28. 7.53	170/110	—	+++	6th to 15th day (no urine)	Diuresis commenced 11.5.53, after which he became hypertensive and convulsed several times during the ensuing week. Gradual improvement then occurred and on discharge he was clinically well, but blood pressure was 130/94 and he still had some proteinuria and hæmaturia.

The graphs were drawn and the peaks for individual fractions were extrapolated to symmetrical Gaussian curves. The relative concentrations of the components were obtained by means of the planimeter (Grassmann, Hannig and Knedel, 1951; Griffiths, 1953).

The total protein concentration of the serum was estimated by the copper sulphate specific gravity method (Phillips, Van Slyke, Hamilton, Dole, Emerson and Archibald, 1950).

RESULTS

Studies were made on five groups of patients: (*a*) Six children with acute glomerulonephritis associated with anuria; (*b*) six children with acute glomerulonephritis without anuria; (*c*) three children with Ellis type II nephritis; (*d*) one child with sulphonamide anuria; (*e*) a control group of five children suffering from scarlet fever without nephritis, and six children suffering from streptococcal sore throats.

The case histories and serum protein analyses are summarized in Tables I to VIII.

Acute Glomerulonephritis with Anuria

In these six patients (Table I) a severe initial acute nephritis was associated with virtually complete anuria for periods ranging from two and a half to thirteen days. The patients were all critically ill in this phase, and a clinical appraisal of the severity of their respective illnesses would place them roughly in the order M.H., C.J. and B.A.M.; F.B. and P.B. B.K.M. was not seen at this stage. With the exception of M.H., who died shortly after the commencement of her diuresis, the patients in this series made quite good clinical recoveries, though in the cases of C.J. and B.A.M. the recovery period was protracted over several months.

The changes in the serum electrophoretic patterns in these patients are pronounced, and seem to be fairly constant and characteristic (Table II). The main features are:

(i) Total protein content was normal or high in the period of anuria, with a further rise coincident with the onset of diuresis, and a gradual fall towards normal in the recovery

TABLE II

Serum Protein Fractions (Grammes per 100 Millilitres): Eight Normal Children and Ellis Type I Nephritis with Anuria

	Date	Total Protein Content	Albumin	Alpha$_1$ Globulin	Alpha$_2$ Globulin	Beta Globulin	Gamma Globulin	Total Globulin Content	Albumin/ Globulin Ratio
Normal serum (eight children)	---	6·3–7·3	4·5–5·0	0·1–0·2	0·5–0·7	0·4–0·6	0·7–1·2	1·7–2·7	1·7–2·6
M.H.	15. 6.53	7·4	2·8	0·3	0·9	0·7	2·7	4·6	0·61
	17. 6.53	7·7	2·9	0·3	0·6	0·5	3·3	4·8	0·60
	18. 6.53	7·9	3·4	0·2	0·4	0·5	3·4	4·5	0·75
	23. 6.53	8·6	3·5	0·1	0·6	0·2	4·2	5·1	0·69
	26. 6.53	8·4	2·5	0·2	0·5	0·6	4·6	5·9	0·42
	30. 6.53	7·8	2·6	0·3	0·8	0·9	3·2	5·2	0·50
C.J.	26. 5.53	7·8	5·0	0·2	0·6	0·3	1·7	2·8	1·79
	11. 6.53	7·5	3·4	0·2	0·9	0·5	2·5	4·1	0·83
	26. 6.53	11·5	4·8	0·2	1·2	1·1	4·2	6·7	0·72
	9. 7.53	9·0	4·4	0·4	1·1	0·8	2·3	4·6	0·94
	23. 7.54	7·0	4·6	0·2	0·8	0·6	0·8	2·4	1·91
	30. 7.54	7·0	4·5	0·2	0·8	0·7	0·8	2·5	1·80
B.A.M.	23. 8.54	8·2	3·9	0·2	0·8	0·6	2·7	4·3	0·91
	25. 8.54	8·1	3·6	0·1	1·1	0·5	2·8	4·5	0·80
	3. 9.54	7·5	2·5	0·1	1·2	0·9	2·8	5·0	0·50
	17. 9.54	7·6	2·6	0·2	1·1	0·6	3·1	5·0	0·52
	7.10.54	6·9	3·6	0·3	0·6	0·5	1·9	3·3	1·09
	21.10.54	7·8	4·1	0·2	0·9	0·7	1·9	3·7	1·11
	7. 1.55	7·9	4·7	0·3	0·7	0·6	1·6	3·2	1·47
F.B.	12. 7.54	6·9	4·3	0·2	0·6	0·3	1·5	2·6	1·65
	14. 7.54	6·8	4·1	0·3	0·4	0·2	1·8	2·7	1·52
	16. 7.54	7·8	3·0	0·5	1·0	0·4	2·9	4·8	0·62
	29. 7.54	7·7	3·2	0·4	0·8	0·7	2·6	4·5	0·71
P.B.	25. 8.53	7·2	3·3	0·8	1·2	0·6	1·3	3·9	0·86
	27. 8.53	7·4	3·4	0·8	1·0	0·8	1·4	4·0	0·87
	31. 8.53	7·5	3·6	0·2	0·5	0·9	2·3	3·9	0·92
	4. 9.53	8·2	4·0	0·5	0·8	1·2	1·7	4·2	0·96
	14. 9.53	7·8	4·2	0·3	0·8	0·7	1·8	3·6	1·19
	21. 9.53	7·3	3·3	0·3	1·0	0·9	1·8	4·0	0·9
B.K.M.	9. 7.53	8·2	4·0	0·4	0·8	0·7	2·3	4·2	0·95

phase. Among the survivors the total protein content was within the normal range at the time of discharge from hospital.

(ii) Albumin content in general was found to be normal or below normal in the anuric phase, with a clear tendency to fall to lower levels with the onset of diuresis, this fall being associated with the loss of albumin in the urine at this time. With clinical improvement of the surviving patients the albumin levels showed a rise towards normal, but in all cases the rise was very gradual and the levels were still subnormal at the time of discharge from hospital. In the cases of B.A.M. and C.J., in which longer follow-up studies were possible, the serum albumin levels were normal in four and twelve months respectively.

(iii) No very significant deviation from normal was manifest in the α_1 globulin levels, though there was a tendency in some cases for the levels to rise slightly after the anuric episodes.

(iv) The α_2 globulin level was found to be higher than normal during the period of anuria, and with the onset of diuresis the high level was maintained or showed a further increase. At the time of discharge from hospital this fraction was still elevated, and in B.A.M. and C.J. the levels were still high normal after four and twelve months respectively.

TABLE III

Essential Features of Case Histories of Children with Acute Glomerulonephritis not Complicated by Anuria

Initials	Age	Sex	Onset of Preceding Infection (Days Before Admission)	Onset of Acute Nephritis (Days Before Admission)	Date of Admission	Date of Discharge	Maximum Blood Pressure, Systolic and Diastolic	Œdema	Proteinuria	Oliguria or Anuria (from Onset of Acute Nephritis)	Progress
N.D.S. ..	13	F.	21	2	5.11.54	26.11.54	160/115	++	+	—	Condition gradually improved and œdema and proteinuria cleared after seven and 14 days respectively. Discharged clinically well, but with a blood pressure of 140/85 and minimal hæmaturia.
R.M.McM.	9	M.	21	7	14. 7.54	28. 7.54	190/150	+	+	—	Condition gradually improved. Discharged clinically well. Blood pressure normal, but with some albuminuria—he was well one month later.
K.S. ..	7	F.	21	9	6. 7.54	13. 7.54	210/140	++	++	—	Admitted convulsing and blind. Blood pressure 210/140, with early bilateral papilloedema and an enlarged heart. Rapidly responded to sedation and a magnesium sulphate enema. Discharged clinically well, normotensive, but with some proteinuria and hæmaturia. She was well three days later.
F.P. ..	11	M.	35	10	16. 7.54	7. 9.54	150/110	+	+++	—	Severe proteinuria and hæmaturia persisted for 21 days and blood pressure gradually returned to normal. Clinical improvement was slow and gradual. Discharged well but with minimal hæmaturia.
L.M.S.	13	F.	21	7	1.10.54	8.10.54	138/110	+	++	—	Condition improved and discharged clinically well, with no œdema, with blood pressure 110/75 but with minimal proteinuria and hæmaturia. Well four months later and urine clear
M.R.B.	3	F.	28	3	18.10.54	27.10.54	125/90	+	++	—	Condition improved and discharged clinically well with no œdema or proteinuria. Blood pressure 110/75 but with minimal hæmaturia.

TABLE IV

Serum Protein Fractions (Grammes per 100 Millilitres) : Ellis Type I Nephritis without Anuria

	Date	Total Protein Content	Albumin	Alpha$_1$ Globulin	Alpha$_2$ Globulin	Beta Globulin	Gamma Globulin	Total Globulin Content	Albumin/ Globulin Ratio
N.D.S.	8.11.54	6·7	3·0	0·3	0·8	0·6	2·0	3·7	0·81
	10.11.54	6·7	3·0	0·3	0·7	0·8	1·9	3·7	0·81
	15.11.54	7·6	3·8	0·1	0·8	0·8	2·1	3·8	1·0
	17.11.54	7·8	3·8	0·2	0·7	0·7	2·4	4·0	0·95
	19.11.54	8·0	3·9	0·1	0·8	0·7	2·5	4·1	0·95
	22.11.54	7·0	4·0	0·1	0·6	0·4	1·9	3·0	1·33
R.M.McM.	16. 7.54	6·6	3·3	0·3	0·6	0·7	1·7	3·3	1·01
	19. 7.54	7·0	3·5	0·3	0·9	0·5	1·8	3·5	1·00
	23. 7.54	7·8	3·8	0·5	1·0	0·6	1·9	4·0	0·95
	10. 8.54	7·8	3·8	0·4	1·0	0·8	1·8	4·0	0·95
	19. 8.54	7·4	4·4	0·1	0·6	0·7	1·6	3·0	1·5
K.S.	7. 7.54	8·2	3·2	0·4	0·5	0·7	3·4	5·0	0·64
	9. 7.54	8·0	3·3	0·4	0·9	0·6	2·8	4·7	0·70
	12. 7.54	10·2	3·6	0·5	1·0	0·9	4·2	6·6	0·55
	16. 7.54	8·6	3·4	0·2	0·7	0·9	3·4	5·2	0·65
F.P.	23. 7.54	8·4	3·5	0·4	0·9	0·7	2·9	4·9	0·71
	29. 7.54	8·7	3·8	0·2	1·0	0·7	3·0	4·9	0·78
L.M.S.	4.10.54	7·2	3·9	0·3	1·0	0·6	1·4	3·3	1·18
	8.10.54	8·4	4·9	0·3	0·8	0·9	1·5	3·5	1·40
M.R.B.	20.10.54	7·5	4·0	0·1	0·6	0·5	2·3	3·5	1·14
	27.10.54	7·5	4·3	0·1	0·8	0·8	1·5	3·2	1·35

TABLE V

Essential Features of Case Histories of Children with Subacute Glomerulonephritis (Ellis Type II)

Initials	Age	Sex	Onset of Subacute Nephritis (Days Before Admission)	Date of Admission	Date of Discharge	Maximum Blood Pressure, Systolic and Diastolic	Œdema	Proteinuria	Oliguria or Anuria (from Onset of Subacute Nephritis)	Progress
B.L.S.	7	M.	14	28. 7.54	15.11.54	130/90	+++	+++	Oliguric for 3½ months	Admitted with gross abdominal distension with ascites, hepatomegaly and splenomegaly. Blood urea within normal range. Liver function tests normal. Urine chromatography showed a general amino-aciduria in the pathological range. Remained oliguric and œdematous for 3½ months with low serum proteins and low albumin/globulin ratio. Then treated with Diamox with diuresis and clearing of œdema and ascites. Discharged improved, but with gross proteinuria and very low serum proteins.
L.A.S.	1	M.	14	17.10.54	8.11.54	110 systolic	+++	+++	Oliguric 11th to 18th days	Diuresis on 21.10.54. Weight fell from 25 pounds (11·4 kilograms) to 20½ pounds (9·3 kilograms) and œdema subsided. Discharged improved without œdema or proteinuria
P.S.G.	5	M.	3 years (nine prior hospital admissions)	18.11.54	8.12.54	120/90	+++	+++	—	Present admission arranged because of increasing œdema and ascites. Blood urea within normal range. Two subsequent hospital admissions and present condition unchanged.

TABLE VI

Serum Protein Fractions (Grammes per 100 Millilitres) : Ellis Type II Nephritis

	Date	Total Protein Content	Albumin	Alpha$_1$ Globulin	Alpha$_2$ Globulin	Beta Globulin	Gamma Globulin	Total Globulin Content	Albumin/ Globulin Ratio
B.L.S.	7.10.54	3·3	0·9	0·2	1·2	0·4	0·6	2·4	0·37
	12.11.54	3·6	1·1	0·1	1·0	0·5	0·9	2·5	0·44
L.A.S.	4.11.54	4·3	1·6	0·5	1·0	0·8	0·4	2·7	0·59
P.S.G.	19.11.54	3·6	0·5	0·1	1·8	0·8	0·4	3·2	0·16

TABLE VII

Serum Protein Fractions (Grammes per 100 Millilitres) : Sulphonamide Anuria

	Date	Total Protein Content	Albumin	Alpha$_1$ Globulin	Alpha$_2$ Globulin	Beta Globulin	Gamma Globulin	Total Globulin Content	Albumin/ Globulin Ratio
M.A.H.	5.10.53	7·1	4·1	0·3	0·9	0·7	1·1	3·0	1·36
	7.10.53	7·7	4·3	0·4	0·9	1·0	1·1	3·4	1·26

TABLE VIII

Serum Protein Fractions (Grammes per 100 Millilitres) : Scarlet Fever Control Cases

	Date	Total Protein Content	Albumin	Alpha$_1$ Globulin	Alpha$_2$ Globulin	Beta Globulin	Gamma Globulin	Total Globulin Content	Albumin/ Globulin Ratio
C.C.	16. 3.55	7·2	4·3	0·2	0·5	0 9	1·3	2·9	1·53
	25. 3.55	7·5	4·9	0·2	0·5	0·6	1·3	2·6	1·85
A.O'S.	16. 3.55	7·4	4·4	0·2	0·8	0·8	1·2	3·0	1·48
	25. 3.55	7·2	4·5	0·2	0·7	0·8	1·0	2·7	1·67
D.B.	16. 3.55	7·9	4·2	0·2	1·0	1·2	1·3	3·7	1·13
	25. 3.55	7·3	4·5	0·2	0·7	0·6	1·3	2·8	1·61
M.D.	16.3. 55	7·2	4·7	0·05	0·6	0·7	1·2	2·55	1·86
M.M.	16. 3.55	8·0	5·0	0·2	0·7	0·8	1·3	3·0	1·70

(v) The β globulin levels did not show any very significant or characteristic changes. There was a tendency for them to be slightly lower than normal in the anuric phase, and to rise gradually in the recovery phase.

(vi) In these studies γ globulin was the serum protein fraction which showed the most characteristic and constant changes. At the times when these patients first came under observation (during their anuric periods) the γ globulin levels were all considerably elevated, and a further rise occurred as they were followed through the course of the anuria. Early in the diuretic phase the γ globulin levels were still steeply rising, but after a variable period following the end of anuria, ranging from three days to four weeks, there was a sharp fall in the levels of this fraction in the surviving patients. In all cases the γ globulin levels were still at least twice the normal value at the time of discharge from hospital. In Figure I the γ globulin levels have been plotted against a time scale in which the end of the anuric phase is used as a common reference point for purposes of comparison. The curves have been extrapolated back to normal at the time in the respective case histories of the onset of the original infection preceding the development of the signs and symptoms of acute nephritis. Whether this extrapolation should have been as a straight line back to the preceding infection, or whether the γ globulin rise occurred predominantly after the onset of the symptoms of acute nephritis could not be determined. In view of the negligible γ globulin increases associated with scarlet fever and streptococcal sore throats in the control series it is probably more likely that the extrapolation should have been to the normal level at the time of onset of the acute nephritis. It is interesting to correlate the shapes of these γ globulin curves with the clinical severity of the disease in the several patients studied.

M.H., the one patient in the series who died, had a serum γ globulin level of 4·6 grammes per 100 millilitres (about five times the normal figure) at the end of the anuric episode, and this was the highest γ globulin level observed throughout the course of investigation. Following the onset of diuresis in this patient the γ globulin level fell sharply, but was still 3·2 grammes per 100 millilitres when last estimated, four days before death.

Of the anuric patients who recovered, the highest γ globulin levels observed were in C.J. (4·2 grammes per 100 millilitres four weeks after the end of the anuric period) and in B.A.M. (3·1 grammes per 100 millilitres eighteen days after the end of the anuric period) and these two patients had clinically a more severe illness than F.B. and P.B., in whom the highest levels recorded were 2·9 and 2·3 per 100 millilitres respectively.

(vii) Total globulin levels in these patients varied in much the same fashion as their main component, γ globulin. They were high in the anuric phase and rose still further in the period of diuresis—although then falling, they were still elevated much above normal at the time of discharge from hospital.

(viii) Albumin/globulin ratios in these patients were low when first observed in the anuric episodes, and subsequently fell much lower (the lowest level being 0·42 in M.H.) before commencement of a very gradual rise towards normal. They were all below normal at the time of discharge from hospital, and B.A.M. still showed an albumin/globulin ratio of only 1·47 four and a half months after the end of his anuric episode.

Although only one serum electrophoretic pattern was obtained in the case of B.K.M., it is included as further evidence of the abnormalities that persist two months after the end of anuria. Total globulin (4·2 grammes per 100 millilitres) and γ globulin (2·3 grammes per 100 millilitres) were still elevated, and the albumin/globulin ratio was 0·95.

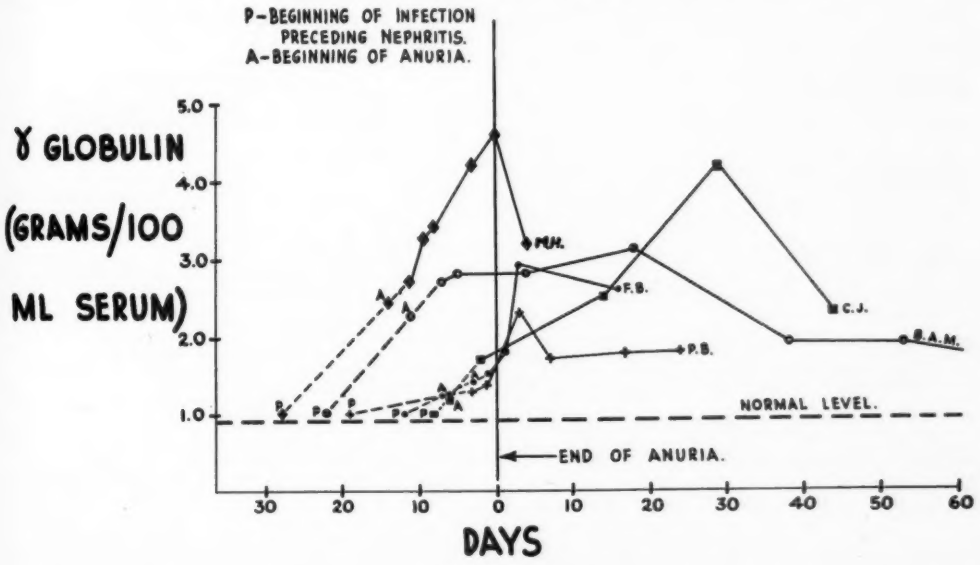

FIGURE I

Gamma globulin levels in patients with acute nephritis and anuria. The curves have been extrapolated back to normal at the time of onset of the acute infection

Acute Nephritis Without Anuria

The six cases of Ellis Type 1 nephritis followed a fairly typical course (Table III).

The main features of the serial electrophoretic studies were as shown in Table IV.

(i) Total protein content was normal or slightly reduced at the time of admission in all cases except that of K.S. (who presented with a hypertensive encephalopathy), but rose to high or high normal levels coincident with the patient's clinical improvement. There was then a gradual change towards normal figures in about two weeks in the cases in which follow-up studies were possible.

(ii) Albumin levels were considerably reduced in all cases at the time of admission to hospital and for several days thereafter, but rose

gradually towards normal levels with the patient's clinical improvement and with reduction in the degree of albuminuria. At the time of discharge from hospital the figures were still below normal, but in the case of R.M.McM., from whom a specimen was obtained at that time, the level was normal three weeks after discharge from hospital.

(iii) The α_1 globulin levels showed no constant deviation from normal and the deviations which were observed were slight and of little significance.

levels, though in all cases the figures were still above normal throughout the period of study, which did not exceed four weeks. In general it will be seen that the γ globulin levels attained in these patients were lower than those in the series with associated anuria, although in the case of K.S. the highest figure attained was 4·2 grammes per 100 millilitres six days after admission to hospital.

(vii) Total globulin levels in all these cases were high at the time of admission to hospital, and continued to rise with a maximum value,

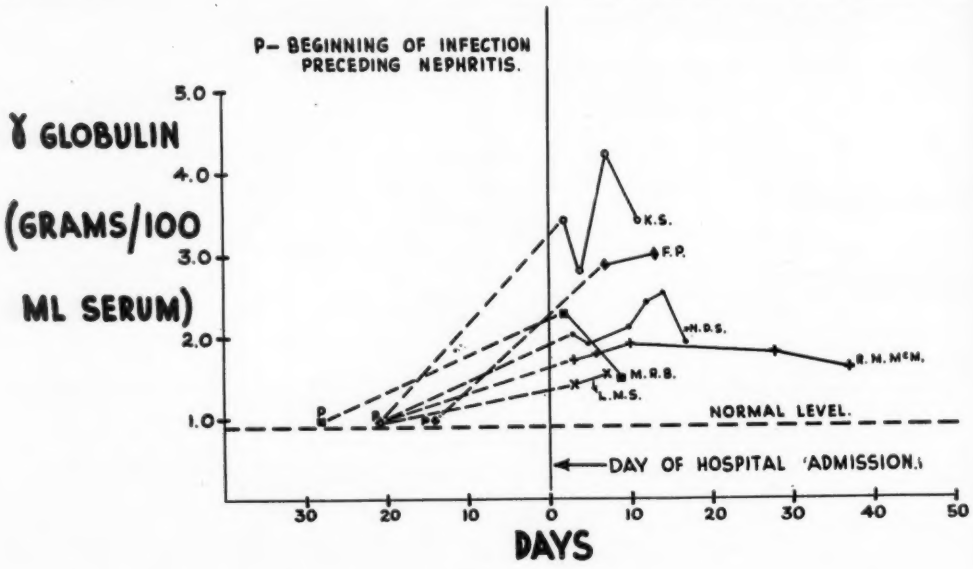

FIGURE II

Gamma globulin levels in patients with acute nephritis without anuria. The curves have been extrapolated back to normal at the time of onset of the acute infection

(iv) The α_2 globulin levels were high on admission, with a tendency towards further rise with the peak occurring at the point where clinical improvement of the patients was beginning. From that point there was a gradual decline towards normal figures.

(v) The β globulin levels were low on admission to hospital, but rose gradually with clinical improvement, usually to levels above normal, before finally reaching and maintaining a level within the normal range.

(vi) In Figure II γ globulin levels are presented in graphical form, the day of hospital admission being used as the reference point for comparative purposes. In all cases the γ globulin level was high on admission, and rose further as the clinical condition began to improve. As clinical improvement further progressed, there was then a gradual decline towards normal

usually coincident with the point of the commencement of clinical recovery. The fall in total globulin content towards normal values then proceeded fairly rapidly, though at the time of discharge the values obtained were still in the region of 3·0 to 3·5 grammes per 100 millilitres.

(viii) Albumin/globulin ratios were low on admission in all cases, and, except in the case of K.S. in which the ratio fell still lower to 0·53 before rising, there was thereafter a gradual rise associated with clinical improvement. Normal values were not attained by the time these patients were discharged from hospital.—

Ellis Type II Nephritis

Serum electrophoretic studies were under taken on three patients with Ellis Type II nephritis (Table V) in order to compare their

patterns with those obtained in the acute nephritic cases which were the main field of study in this investigation.

Total protein content in all cases was very low (Table VI), owing to a gross reduction in the level of the albumin fraction, which was reduced to about one-quarter of the normal figure. Total globulin levels were normal, but there was an alteration in the various globulin components with a great increase in the α_2 fraction to twice or three times the normal level and a fall of much the same magnitude in the γ globulin fraction. The very low albumin levels and the normal total globulin levels resulted in very low albumin/globulin ratios (0·16–0·59).

Sulphonamide Anuria

M.A.H., aged two years and six months, was admitted to hospital on October 4, 1953. Two days previously he had ingested 10 sulphonamide tablets (3·0 grammes). The day before admission he vomited and complained of pains in the legs. He passed no urine for thirty-six hours before admission.

When seen in hospital his bladder was catheterized and 30 millilitres of bloodstained urine were obtained—no sulphonamide crystals were detected. Cystoscopy and ureteric catheterization were performed the next day and blood clot and crystals were washed out of the renal pelvis. He passed urine normally the following day (that is after three and a half days of anuria) and continued to pass urine normally thereafter.

Serum electrophoresis was performed on two occasions, once during his period of anuria and once the day after he recommenced passing urine (Table VII).

Although this child was anuric for a total period of three and a half days, and serum electrophoresis was performed towards the end of this period, and again two days later, the patterns show little if any deviation from the normal range. The α_2 globulin fraction alone (0·9 gramme per 100 millilitres) shows an abnormality, and this is reflected in a slightly higher than normal total globulin level and a slightly low albumin/globulin ratio. The two patterns obtained are essentially similar, a finding which is quite different from that in our cases of anuria associated with acute nephritis.

Control Cases

Electrophoretic studies were performed on the sera of eleven children of a similar age group to the series studied. Five of these children had scarlet fever treated with penicillin eight days before the first electrophoretic pattern was estimated, and three of them had a further estimation nine days later. The other six children had streptococcal sore throats (two each of type IV and type VIII, one type VI and one type XII) for which they had no specific treatment or were treated with sulphonamide only. Electrophoretic patterns were performed on this latter group of six children four weeks after the onset of their symptoms. The electrophoretic patterns of two of the children with scarlet fever were normal at eight days and were not repeated. The other three children showed β and γ globulin values at about the upper limit of normality with low normal or low albumin/globulin ratios, but when reassessed nine days later their patterns were all essentially normal (Table VIII). The six children with a history of bacteriologically proven streptococcal sore throats all had electrophoretic patterns showing little or no deviation from normal. In three of these children the α_2 and β globulin contents were slightly elevated, the highest values being 0·9 and 0·8 gramme per 100 millilitres respectively. In all six cases γ globulin values were normal or very slightly elevated, with a range of 1·0 to 1·4 grammes per 100 millilitres. These patterns, taken at varying times up to four weeks after the onset of illnesses of a similar type to those preceding the attacks of acute nephritis in the series presented, in no way resemble the patterns obtained in the nephritic children studied. It would seem reasonable therefore to exclude the preceding infection as a possible cause of the abnormal patterns found.

DISCUSSION

The present study was undertaken in an attempt to correlate the serum protein changes in acute nephritis with the various phases of the clinical course of the disease.

The two main groups studied were Ellis Type I with or without anuria. The serial serum electrophoretic patterns in these two groups resembled each other in the several deviations from normal, but showed quite pronounced differences in the degree of deviation. Generally there was a rise in the total protein concentration, to a level about 20% above normal. In the cases with associated anuria this rise occurred mainly in the diuretic and early recovery phase, while in the less severe cases without anuria the rise occurred gradually into the early recovery period. Patients followed over a longer period showed a subsequent total protein fall to normal figures with further clinical improvement. This fall to normal values may occur quite rapidly over a period of several days.

The most significant variations from normal were found in the case of albumin and of γ globulin. Albumin level was initially low in

both groups, the values being about 30% below normal. In the group of anuric patients, however, there was a further sharp fall at the beginning of the diuretic phase undoubtedly associated with the loss of albumin in the urine beginning at this time. This further fall did not occur in the less severe non-anuric group, the level remaining fairly constant for several days. At the commencement of clinical improvement in both groups of patients there was an associated rise in the serum albumin levels, continuing throughout the recovery period until normal values were attained in two or three weeks.

The greatest and most prolonged changes were in concentration of γ globulin. The increase in γ globulin above normal values ranged from 70% to about 450%, with a clear correlation between the magnitude of the rise and the clinical severity of the condition. The more severe cases showed a rise in γ globulin to 250% to 450% above normal values, while in the less severe cases the rise was 70% to 300% with the greater number of cases in the lower range. The return of γ globulin levels to the normal range was more gradual than the return of either total protein or albumin levels, and B.K.M. at two months, and B.A.M. at four months after the onset of the illness still showed levels respectively two and a half times and twice the normal value.

The cases of Ellis Type II nephritis gave electrophoretic patterns which were quite different from those obtained in the acute nephritis. Their most significant feature was a uniformly very low albumin level and a raised α_2 globulin level, a finding which confirms the figures of MacKay, Volwiler and Goldsworthy (1954) and of Steger (1954) in the nephrotic stage of glomerulonephritis. This inverse relationship of albumin and α_2 globulin levels was also noticed by other workers including Grassmann, Hanig and Knedel (1951), Bongiovanni and Wolman (1949), and Leutscher (1941).

These findings resemble those of Jayle *et alii* (1954), who demonstrated similar low albumin and high γ globulin levels in acute nephritis, and low albumin and high α_2 globulin levels in subacute nephritis. Their estimations were apparently carried out early in the respective illnesses, and were not repeated through the disease.

The one case of sulphonamide anuria is included in the cases studied as the patient's electrophoretic pattern was essentially normal on two occasions. This fact suggests that the abnormal patterns obtained in the cases of acute nephritis with associated anuria were not merely the result of the anuric state, but were due to its causative clinical condition.

The electrophoretic patterns in the eleven control cases of scarlet fever and streptococcal sore throat showed little deviation from normal, and any globulin fraction rise was minimal. Care was taken that all the nephritic patients were free of intercurrent infection at the times when the sera were taken, and throat swabs were uniformly " negative ". For these reasons it may be concluded that the γ globulin rise found throughout the acute nephritic studies was a function of the acute nephritis and not merely a manifestation of either the preceding or of any intercurrent infection.

Further work is in progress on the serological findings of the cases presented in this study. It has been suggested by Lange, Gold, Weiner and Simon (1949), Bialestock (1951, 1953), Lange, Weiner, Gold, Tchertkoff and Simon (1949), Vorlaender (1952), Sarre (1952) and Sarre and Rotner (1954) that there is an auto antibody production in glomerulonephritis. The gross increase in γ globulin which occurs in the early stages of this disease would certainly fit in with such an hypothesis, and it is hoped that the further serological work in progress may throw some light on the problem.

SUMMARY

Serial serum electrophoresis studies throughout the course of their illnesses have been carried out on fifteen children suffering from acute glomerulonephritis (with and without anuria) and subacute glomerulonephritis. The electrophoretic patterns have been compared with patterns obtained from eight normal children, one child with sulphonamide anuria, five children with scarlet fever and six children with bacteriologically proven streptococcal sore throats.

Certain of the alterations in the serum protein fractions have been found to be fairly constant and characteristic, and to bear a definite relationship to the clinical severity of the disease.

In acute glomerulonephritis there was a rise in total serum protein concentration of about 20%. In the more severe cases with associated anuria the main rise occurred in the diuretic and early recovery phase, while in the less severe non-anuric cases the rise was more gradual and ran into the period of beginning recovery. In each instance there was a subsequent fall to normal levels with further clinical improvement.

Serum albumin levels were low in acute glomerulonephritis, and showed a further sharp fall in the early diuretic phase in cases with associated anuria.

Of all the protein fraction changes in acute glomerulonephritis, the greatest and most prolonged occurred in the concentration of γ globulin. There was a rise of 70% to 450% above normal values, with a clear correlation between the magnitude of the rise and the clinical severity of the disease. The highest γ globulin levels occurred at the point of beginning clinical recovery. The return to normal values of γ globulin was more protracted than the return of total protein or albumin concentrations.

Cases of subacute glomerulonephritis showed quite different serum electrophoretic changes, the most significant features being uniformly very low albumin levels and raised α_2 globulin levels.

The serum electrophoretic patterns in the case of sulphonamide anuria were essentially normal in all respects, while the patterns following scarlet fever and streptococcal sore throats were either normal or showed minimal deviations.

The significance of these findings is discussed in relation to the severity and clinical course of the diseases.

ACKNOWLEDGEMENTS

But for the interest and cooperation of the Board and Medical Staff of the Royal Children's Hospital, Melbourne, this study would not have been possible. It is with particular pleasure, therefore, that we record our thanks to the senior medical staff for allowing us access to the patients and the resident medical staff for their cooperation, and in particular to Dr. V. L. Collins, Dr. H. E. Williams, Dr. W. B. Macdonald and Dr. M. J. Robinson, and also to Mr. Ian Jack. Our thanks are also due to Dr. H. McLorinan and Dr. Margaret Holmes, of the Fairfield Infectious Diseases Hospital, Melbourne, for access to and information concerning the control cases of scarlet fever and streptococcal sore throats, to Dr. R. N. Mellor, of the Mildura Base Hospital, Victoria, for follow-up information and serum samples from one patient in the series, and to Professor R. Douglas Wright for his interest and encouragement, and his helpful advice through all stages of this investigation.

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DIRECT EFFECTS OF RESERPINE, RESCINNAMINE AND CANESCINE ON BLOOD VESSELS[1]

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The active principles from plants of the genus Rauwolfia have now come into very widespread use in the treatment of hypertension. Derivatives of the species *Rauwolfia serpentina* have been most widely studied, and amongst these the pure alkaloid reserpine, first isolated by Müller *et alii* (1952), has proved particularly valuable. Its pharmacology has been the subject of numerous reports including those by Bein (1953), by Trapold *et alii* (1954), by Plummer *et alii* (1954), and by McQueen *et alii* (1955).

Reserpine is thought to exert its principal hypotensive action by inhibition of central sympathetic nervous mechanisms. However, there is evidence of a direct peripheral vasodilating effect in perfused innervated blood vessels (McQueen *et alii*, 1955), and also in perfused vessels in which vasomotor tone has been increased by " Pitressin " and by barium chloride (Tripod and Meier, 1954). The present studies have been performed to assess the effect of reserpine on blood vessels in which vasomotor tone has been increased by humoral pressor substances—noradrenaline, " Pitressin " and barium chloride.

Clinical experiences with two further newly isolated alkaloids, rescinnamine from *Rauwolfia serpentina* and canescine (recanescine, deserpidine) from *Rauwolfia canescens*, suggest that these two substances may have a useful place in the therapeutic armamentarium against hypertension (Smirk and McQueen, 1955).

In the present communication we compare their effects on perfused blood vessels with those of reserpine. One feature of the direct effect of reserpine is its prolonged duration. We have studied the duration of action of all three by means of the innervated rat diaphragm preparation, on which the alkaloids have been found to have an inhibitory effect.

[1] Received on September 19, 1955.

Methods

The animals used were white rats of the Wistar strain, aged from seven to nine months. The constant-rate perfusion rat hindquarter preparation used was described by Fastier and Smirk (1947). The rats were anæsthetized with ether and the abdominal aorta was cannulated, after which the animal was bisected and the lower half pithed. The hindquarter was perfused with Krebs-Henseleit Ringer solution incorporating 6% dextran of molecular weight approximately 70,000. Oxygen with 5% carbon dioxide was bubbled through this solution for an hour before and during the experiment, the final pH being 7·4. A constant-output rotary pump was used in this preparation and variations in vasomotor tone were measured by changes in the pressure within the perfusion circuit. A perfusion rate of three millilitres per minute was used in all the rat hindquarter experiments. When " Pitressin " and barium chloride were employed for the elevation of vasomotor tone they were added direct to the medium. When noradrenaline was used, it was introduced via a needle into the rubber tubing connected to the cannula by means of an electrically driven syringe.

For the purpose of comparing the duration of action of the three alkaloids, the innervated rat diaphragm preparation described by Bülbring (1946) was used. The animals were killed by a blow on the head and the left side of the diaphragm and intact phrenic nerve were removed and mounted in a glass organ bath of 50 millilitres capacity. This contained Krebs-Henseleit Ringer solution, through which 95% oxygen and 5% carbon dioxide were constantly bubbled, and was maintained at a temperature of 36° to 37° C. Electrical stimuli were applied to the phrenic nerve at a rate of nine impulses per minute, square waves of two milliseconds' duration and sufficient intensity to produce maximal contraction being used. Stimuli were also applied directly to the muscle.

RESULTS

Antagonism to Pressor Effects of Noradrenaline, "Pitressin" and Barium Chloride

Figure I shows the effect of 0·2 milligramme of reserpine on the perfusion pressure when this had been raised by the continuous injection of noradrenaline at the rate of five micro-

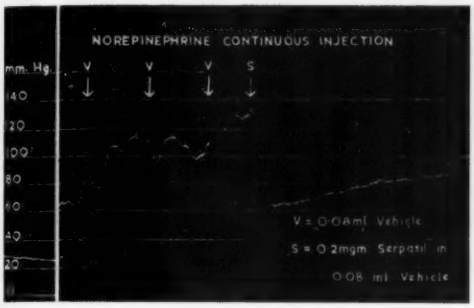

FIGURE I

Rat hindquarter preparation. Constant flow infusion, pressure at left of figure. Pressure raised by continuous injection of noradrenaline with electrically driven syringe at rate of five microgrammes a minute. At *V*, injection of 0·08 millilitre of buffered ascorbic acid vehicle. At *S*, injection of 0·2 milligramme of reserpine in 0·08 millilitre of vehicle

grammes per minute. In the experiment illustrated in Figure II, the perfusion pressure had been raised by the addition of 0·3 millilitre of "Pitressin" (Parke, Davis—20 pressor units per millilitre) to 200 millilitres of the

FIGURE II

Preparation as in Figure I. Pressure raised by addition of 0·3 millilitre of "Pitressin" (Parke, Davis—20 pressor units per millilitre) to 200 millilitres of perfusion medium. At *V*, injection of 0·08 millilitre of buffered ascorbic acid vehicle; at *R*, 0·2 milligramme of canescine in 0·08 millilitre of vehicle; at *S*, 0·2 milligramme of reserpine in 0·08 millilitre of vehicle

perfusion medium. The effect is shown, firstly of canescine, and secondly of reserpine, each 0·2 milligramme. Injection of the buffered ascorbic acid medium, in which they were dissolved, itself produces a considerable fall in perfusion pressure when "Pitressin" is the pressor agent, but it is of considerably less degree and briefer duration than that produced by the canescine or reserpine.

Figure III shows the effect of 0·2 milligramme of rescinnamine on perfusion pressure

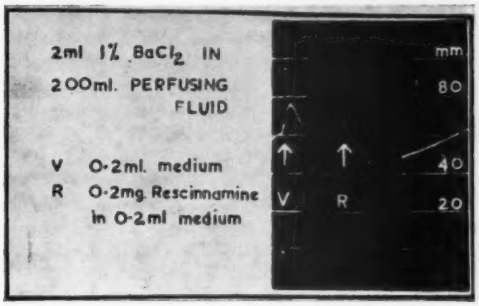

FIGURE III

Preparation as in Figure I. Pressure raised by addition of barium chloride to perfusing fluid. At *V*, 0·2 millilitre of buffered ascorbic acid vehicle; at *R*, 0·2 milligramme of rescinnamine in 0·2 millilitre of vehicle

raised by the addition of 20 milligrammes of barium chloride to 200 millilitres of perfusion medium.

Direct effects against vasoconstriction by each of the humoral agents employed could be demonstrated with very much smaller doses than those illustrated in the previous figures.

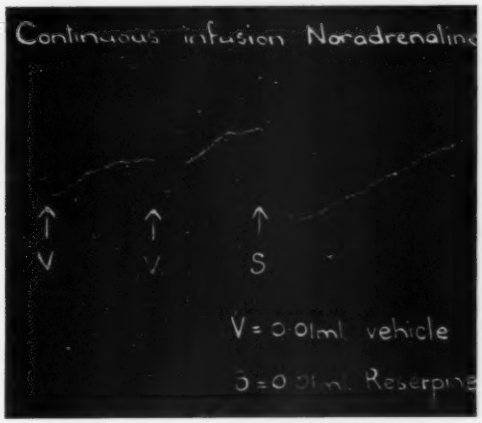

FIGURE IV

Preparation as in Figure I. Pressure raised by continuous injection of noradrenaline, five microgrammes per minute. At *V*, 0·01 millilitre of buffered ascorbic acid vehicle; at *S*, 0·02 milligramme of reserpine in 0·01 millilitre of vehicle

The effect of 0·02 milligramme of reserpine on perfusion pressure raised by continuous injection of noradrenaline is shown in Figure IV. However, the difficulty of maintaining the alkaloids in solution, except in highly acid ascorbate solutions, makes the demonstration of the effects of very small amounts difficult, since they tend then to be overshadowed by those of the medium.

A similar effect was observed when the reverse process was performed. Addition of the alkaloids to the perfusion medium diminished the effect of repeated injections of noradrenaline as shown in Figure V. In this experiment the pressor responses to injections of four microgrammes of noradrenaline are shown before and after the addition of 2·5 milligrammes of canescine to 220 millilitres of perfusion fluid. Not all of this amount remains in solution after addition of the alkaloid in its ascorbate solvent to the perfusion medium, and this is the case with each of the alkaloids. However, the residue appears to be biologically insignificant when tested in turn against " Pitressin " in the rat hindquarter preparation.

One feature of this peripheral effect of the alkaloids is its prolonged duration. The effect of reserpine seems to last longest, that of rescinnamine next, and canescine appears to have the shortest action of the three.

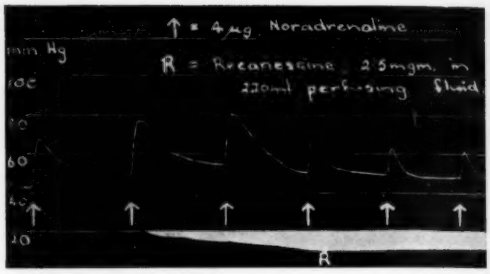

FIGURE V

Preparation as in Figure I. At *R*, 2·5 milligrammes of canescine added to 220 millilitres of perfusing fluid. At arrows, separate injections of four microgrammes of noradrenaline. Shaded area indicates approximate length of time for full concentration of canescine to reach the preparation

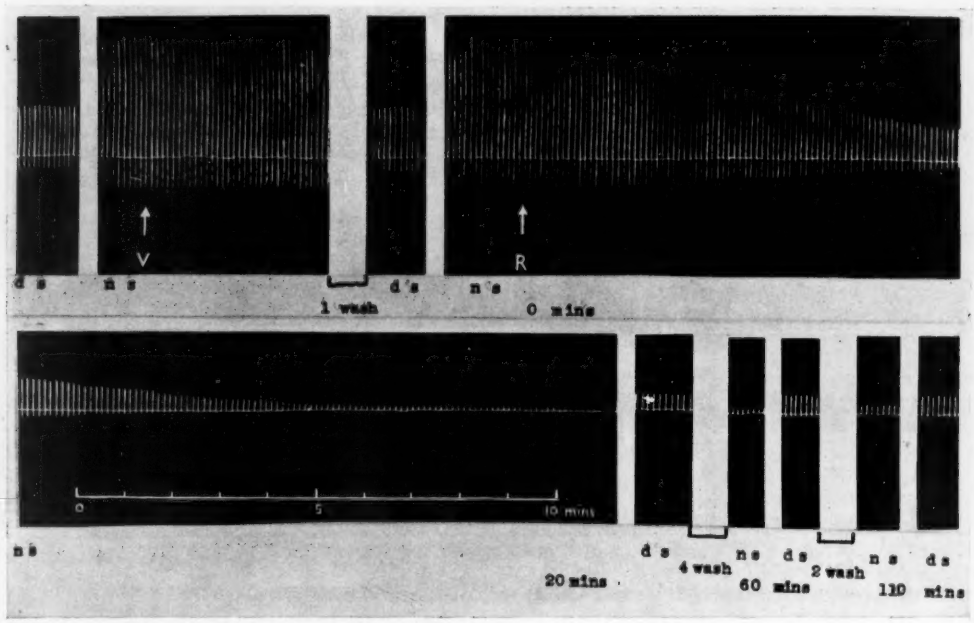

FIGURE VI

Rat diaphragm preparation. Electrical stimuli at the rate of approximately nine impulses per minute. Square waves of two milliseconds' duration ; d.s.=direct stimulus to muscle ; n.s.=stimulus via phrenic nerve. Time course and scale as shown. At points indicated, preparation washed by emptying and refilling organ bath with Krebs-Henseleit Ringer solution. At *V*, addition of 0·4 millilitre of buffered ascorbic acid to water bath. At *R*, addition of 1·0 milligramme of reserpine in 0·4 millilitre of buffered ascorbic acid

Effects on Rat Diaphragm Preparation

The behaviour of the rat diaphragm preparation towards each of the alkaloids is similar. On the addition of 1·0 milligramme of alkaloid in the ascorbic acid-ascorbate medium to the 50 cubic centimetre water bath in which the nerve-muscle preparation is suspended, the magnitude of the contractions progressively diminishes, and if the alkaloids are left in contact with the preparation for a sufficient length of time, contractions are abolished. The action of the medium alone is slight and transient. The principal effect of the alkaloids is on contractions elicited via the nerve. However, a moderate impairment of contraction elicited by direct stimulation of the muscle can be shown. Partial recovery usually occurs after repeated washing, although it is even then incomplete. However, in the case of reserpine, even repeated washing may fail to restore contractions. These effects are illustrated in Figures VI and VII. Again, the introduction of the alkaloid into the Krebs-Henseleit Ringer solution under the conditions of the experiment causes precipitation of some of the alkaloid.

DISCUSSION

It has been consistently reported that, in the intact animal, reserpine does not block the pressor effect of noradrenaline and may indeed enhance it (Bein, 1953; Trapold *et alii*, 1954; Plummer *et alii*, 1954), probably by central inhibition of the reflex compensatory mechanisms which ordinarily limit the rise in blood pressure produced by noradrenaline. Further, in isolated blood vessels, and in amounts they considered to be of the order of those locally active therapeutically (one microgramme), Tripod and Meier (1954) found reserpine to exert no antagonism towards noradrenaline. However, a peripheral vasodilating effect (in the same amounts) was demonstrated by the latter authors on coronary vessels in which the vasomotor tone had been increased by " Pitressin " and barium chloride,

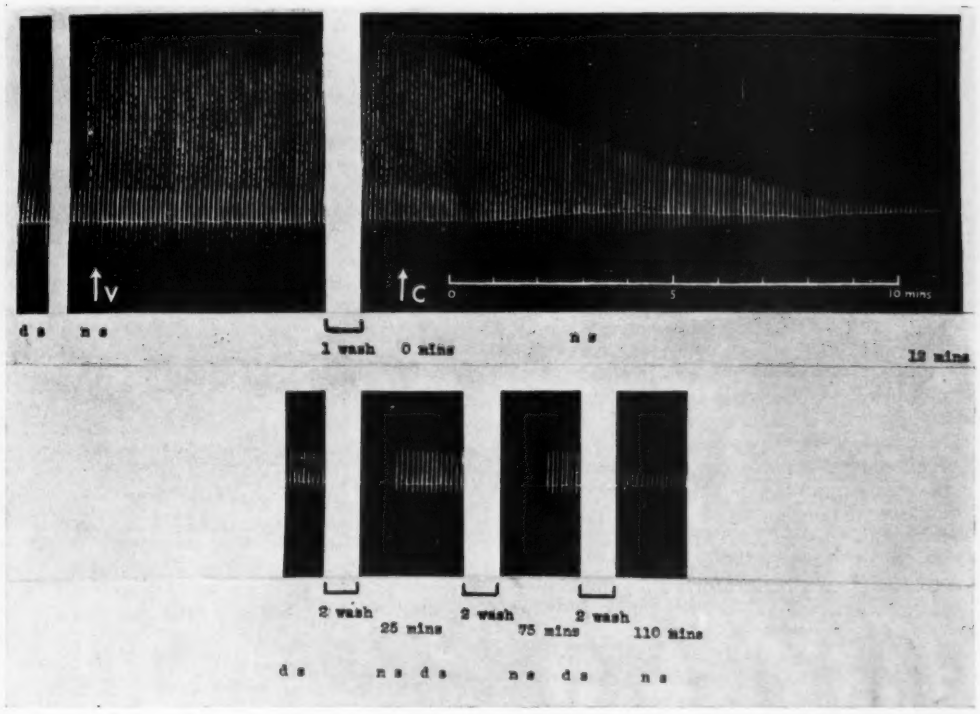

FIGURE VII

Preparation, scale, vehicle *et cetera* as for Figure VI. C=canescine, 1·0 milligramme, in 0·4 millilitre of buffered ascorbic acid

and on the vessels of the isolated rabbit hind limb treated with barium chloride.

The present series of experiments shows that, at least in doses of the order of 20 microgrammes, reserpine has a direct vasodilating effect on rat blood vessels constricted by noradrenaline, as well as by " Pitressin " and barium chloride. Rescinnamine and canescine also have similar effects.

In the rat diaphragm preparation the principal effect of the alkaloids is on contractions elicited via the nerve. However, a moderate impairment of contraction elicited by direct stimulation of the muscle was shown. Whatever the mode of action, the similarity of the effects of the three alkaloids and their prolonged and persistent action are illustrated. Reserpine has been shown to act more slowly than either of the other two, but to have an action which, when it has occurred, is at times not reversible, even by repeated washing. Canescine and rescinnamine act more quickly, but their effects can consistently be removed, at least partially, by washing. It is suggested that the differences in duration and reversibility of their effects are the consequences of differences in solubility of the three compounds. Reserpine is dissolved only with considerable difficulty. Rescinnamine is somewhat more readily soluble, and canescine even more so. It would seem likely, therefore, that reserpine would enter tissues only with difficulty, and be even more difficult to expel, canescine and rescinnamine entering and leaving more readily.

Peculiar persistence of the effects of Rauwolfia alkaloids on isolated smooth muscle has been demonstrated by Bannerjee and Lewis (1955). The amounts of the alkaloids introduced in some of the present series of experiments were of the same order weight for weight (0·1 milligramme per kilogram) as those which have been administered intravenously for therapeutic purposes (Hughes *et alii*, 1955). It is possible, furthermore, that the tendency to persistence of their effects may permit, even in the case of the usual much smaller therapeutic doses, a cumulative action and final clinical attainment of comparable tissue concentrations. The slow development of maximum effect on small doses and the long continuance of its manifestations after cessation of the drug give clinical support to this idea.

SUMMARY

Reserpine and the related alkaloids rescinnamine and canescine have been shown to have a direct vasodilating effect on isolated blood vessels, the tone of which had been increased by noradrenaline, " Pitressin " or barium chloride. The relative duration of the effects of the three alkaloids has been investigated by means of the isolated rat diaphragm preparation, on which they have an inhibitory action. All have a peculiarly persistent effect not completely removable by washing, that of reserpine being slowest in development and most difficult to reverse. It is suggested that these differences are the result of differences in solubility.

ACKNOWLEDGEMENTS

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AN ANALYSIS OF THE VALUE OF MEDICAL CARE IN A GROUP OF REPATRIATION MALE DUODENAL ULCER PATIENTS[1]

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DURING the period 1947–1949 we were given the opportunity of treating a large number of patients suffering from duodenal ulceration in the Repatriation General Hospital, Concord; the subsequent progress of a number of them has been followed over the next five or more years. The patients were all male ex-servicemen.

A series of 105 patients was selected for observation. These represented consecutive admissions, except that those patients with associated gastric ulceration or suffering from cardiac, renal, pulmonary or other complications were excluded. This group, then, represented uncomplicated duodenal ulcer sufferers.

The purpose of this study was to determine the course of the disease in these patients, and to evaluate medical care and the importance of such factors as diet, antacids, tobacco and alcohol consumption, duration of symptoms, incidence of hæmorrhage and perforation, emotional stress and mental state on that course.

The diagnosis of duodenal ulceration was established on undisputed radiological evidence on one or more occasions. In all cases gastric secretory studies revealed high acid values. The men remained in hospital on a standard ulcer regime until "healing" of the ulcer had occurred. Healing was based on the loss of symptoms and disappearance of the ulcer "niche". (We fully realize the limitations of these criteria, but so far as we could judge these patients' ulcers had healed when they left hospital.) They then returned to the care of their own doctors and were not further treated by us unless readmitted to hospital. They were reviewed by us personally at intervals of six to twelve months for the next five to eight years.

Of the original 105 men, five died during the next five years from causes unrelated to their ulcer and have been eliminated from the study. The remaining 100 men were examined regularly and reviewed by us finally during the last six months. There were no deaths due to the ulcer.

[1] Received on September 14, 1955.

The age group in the series ranged from the third to the sixth decade with a mean age of thirty-five years. Fourteen of the men were unmarried or widowers and 86 married. Of the marriages, 70 were reasonably satisfactory and 16 unsatisfactory. The occupations were varied; the patients included labourers, public servants, clerks, salesmen, drivers, farm workers, skilled tradesmen, and transport men. The great majority (92%) were employees.

CRITERIA IN ASSESSMENT OF RESULTS

The basis for assessment of results was made on recurrence of symptoms, development of complications such as hæmorrhage, perforation and stenosis, readmissions to hospital and the average time lost from work in each year.

The results of medical care were classified by these criteria into three groups: group A, satisfactory; group B, failure; group C, intermediate.

The satisfactory group was allowed one hæmorrhage and one readmission to hospital and no more than three weeks' average loss of work per year; the intermediate group was allowed one hæmorrhage, two hospital readmissions and up to six weeks' loss of work. The development of perforation or stenosis, two or more hæmorrhages, and the loss of eight or more weeks' work or recourse to surgery placed that person in the failure group.

The criteria used for classification are summarized in Table I:

TABLE I
Criteria for Classification

Factors	Group A	Group B	Group C
Stenosis	0	0	+
Perforations	0	0	+
Hæmorrhages	0 or 1	1	2 or more
Hospital readmissions	0 or 1	Up to 2	3 or more
Average work time lost for year	3 weeks	6 weeks	8 weeks or more
Symptoms	Free for long periods	Definite free periods	Constant
Surgery	0	0	Gastrectomy

On the basis of these criteria the results of medical care in the 100 cases were as follows :

TABLE II

Results in 100 Cases of Duodenal Ulceration at the Repatriation General Hospital, Concord, Followed for Five to Eight Years

Group	Number of Cases
Group A (satisfactory) ..	35
Group B (recurrent ulceration with healing) ..	17
Group C (failure)	48

DISCUSSION

Long-term follow-up surveys in duodenal ulceration have not appeared in the Australian medical literature, and they have been few in the United Kingdom. Martin and Lewis (1949) stated that they had been " unable to discover any series of cases in this country (United Kingdom) which provides a follow-up survey of ten years and very few elsewhere ". In their series of cases from Addenbrooke's Hospital, Cambridge, there were 105 cases of duodenal ulceration, of which only 62 cases were traced. These patients were mostly interviewed by the authors, but were never under their personal care. Of those that died, approximately 20% did so as a direct result of their ulcer.

Rae and Allison (1953) reported the results of an experimental scheme carried out in the Royal Navy for the rehabilitation and employment of men with healed peptic ulcers. These men were reviewed after five years. There were 47 cases of duodenal ulceration, and of these 21 or 44·7% were judged satisfactory.

In the United States, Flood in 1948 reported 181 males and 52 females followed at the Presbyterian Hospital for an average of 6·9 years, 21% remaining continuously free from significant symptoms. Jordan and Kiefer (1932), having followed the small number of 37 cases for five years, report 46% recurrences, so that presumably 54% were satisfactory.

The best documented series of cases comes from Scandinavia. Natvig *et alii* (1943) report a three-year follow-up investigation of 230 cases of duodenal ulceration from the Drammen Hospital, Norway. At the end of three years only 34% of the patients were free from symptoms. The same material was followed for a period of eight years by Römcke and Qvigstad (1946). It was then found that of the original 230 patients only 26 or 11·3% were free of symptoms.

Krarup (1946) reported from Bispebjerg Hospital, Copenhagen, 246 cases of duodenal ulcer followed for five years, and stated that 19% of the patients recovered, 28% improved, 36% had poor results, 11% died, and 6% were not located.

Malmros and Hiertonn (1949), from the Orebro Central Hospital, Sweden, analysed the results of medical treatment during the period 1936 to 1939 of 495 patients with duodenal ulcer. They found a favourable course in 29%, a less favourable course in 22%, a serious condition in 41%, and deaths from other causes in 8%. In all these Scandinavian series, assessment was made by use of the *questionnaire* form, in our opinion a very serious drawback in the true evaluation of results. Moreover, no criteria were given as to how the classifications were made. We find it difficult to compare our results with any of these series. If we took absence of symptoms as the criterion of cure in our cases, the satisfactory results would have been close to zero.

TABLE III

Group C (Failure Group)

Factor		Number of Cases
Stenosis		7
Perforations		5
Major hæmorrhages (two or more)		20
Admissions to hospital (two or more)		43
Loss of time from work in year :		
2 months	9	
2 to 3 months	15	44
Over 3 months	20	
Gastrectomy		16
Total		48

However, in all these series it is fairly obvious that the results of medical care are far from good, in so far as the proportion of satisfactory results after a number of years is well below 50%.

In the light of these reports our figure of 35% satisfactory appears about average. We should like to point out that we realize that this figure is probably low for the overall cases of duodenal ulceration, and that it represents a figure for patients who required admission to hospital. In the failure group (48%), seven developed pyloric stenosis, five had perforations, 20 had two or more major hæmorrhages, 43 were readmitted to hospital two or more times, and 44 lost two or more months' work in every year. By the time this survey was complete, 16 had had a partial gastrectomy performed and several were awaiting operation (see Table III). The economic importance of this

disability is emphasized by an estimate of the loss of work in man-hours, loss of wages and cost in pensions in this group as set out in Table IV.

Evaluation of the Importance of Duration of Symptoms, Incidence of Hæmorrhage and Perforation, and Various Ætiological Factors

In attempting to evaluate the prognostic significance of the various factors studied, comparison was made between the satisfactory and failure groups on a percentage basis. The

TABLE IV

Group C (Failure Group, 48 Men: Six-Year Period)

Factor	Amount
Loss of work	138,240 man-hours
Loss of wages	£49,440 (basic wage estimate)
Pensions	£59,904

figures are too small to have any statistical significance and are interpreted as showing a trend only.

Duration of Symptoms.—If symptoms had been present for less than two years prior to the commencement of the study, the percentages were equal in the two groups A and C. If the symptoms had been present for up to five years, the percentage in the failure group was considerably higher, and the findings were similar when symptoms had been present for longer periods, up to and over ten years (Table V). These findings suggest that in this series duration of symptoms over five years has a less favourable prognostic outlook.

TABLE V

Relationship between Duration of Symptoms, and the Results

Duration of Symptoms Prior to Commencement of Study	Group A	Group B	Group C	Total
Less than 2 years	4 (44%)	1 (12%)	4 (44%)	9
Less than 5 years	9 (33%)	4 (15%)	14 (52%)	27
5 to 10 years ..	20 } 22	11 } 12	26 } 30	64
Over 10 years ..	2 } (34%)	1 } (19%)	4 } (47%)	

This is in keeping with published reports. Natvig *et alii* (1943), in their three-year follow-up of 230 cases, found that if the duration of symptoms was less than five years the proportion of satisfactory results was 40%, as compared with 25% for a duration over five years. Qvigstad and Römcke (1946), following these cases for eight years, found that the under five years' duration group contained 13·4% in the satisfactory category as compared with 7·6% in the over five years group.

Malmros and Hiertonn (1949), in their survey of 495 cases followed for five years, reported that if the duration of the history exceeded five years, 87·5% were unsatisfactory, while if it was under five years this figure was 63·1%.

Incidence of Hæmorrhage Prior to the Time of Commencement of this Study.—If there had been no major hæmorrhages prior to the time of the study, the percentages in the satisfactory and failure groups were the same (Table VI).

TABLE VI

Relationship between the Number of Major Hæmorrhages Prior to Time of Commencement of Study, and the Results

Number of Hæmorrhages	Group A	Group B	Group C	Total
Nil	24 (44%)	7 (13%)	23 (43%)	54
One	4 (20%)	5 (25%)	11 (55%)	20 (90%)
Two	3 (20%)	3 (20%)	9 (60%)	15
Three	1 } 4	2 } 2	2 } 5	11
Three and over ..	3 } (36%)	0 } (18%)	3 } (46%)	

If one hæmorrhage had occurred, the percentage in the failure group was 45%, compared to 20% in the satisfactory group. With two or three or more hæmorrhages, the percentage figure was significantly higher in the failure group. It would appear, then, that in this series the occurrence of a hæmorrhage gave a worse prognosis. It is nevertheless of interest that there were four patients in the satisfactory group who had had three or more major hæmorrhages prior to the commencement of the study.

The foregoing findings are again in agreement with published accounts in the literature. Jordan and Kiefer (1932) analysed 60 cases of successful treatment of duodenal ulceration, and 60 cases of unsuccessful treatment, and found a tendency to hæmorrhage in 55% of the unsuccessful group and in 15% of the successful group.

Incidence of Perforation Prior to Commencement of the Study.—There were 13 patients who prior to the study had had a perforation of a duodenal ulcer treated by simple closure, and the percentages in the satisfactory and failure groups were almost the same. Although the

figures are small, they suggest that in this series the occurrence of a prior perforation did not materially affect the prognosis (Table VII).

Avery Jones and Doll reported in 1953 a follow-up investigation after two to twelve years of 64 patients with perforated duodenal ulcers, and concluded that the late prognosis was affected by the duration of the history prior to the perforation, and by whether or not the ulcer had been previously diagnosed. They do not state whether they considered that the perforation episode itself had any prognostic value. However, they do stress that if the duration of the ulcer symptoms was long prior to perforation, the prognosis was generally poor.

TABLE VII

Relationship between Perforations Prior to the Commencement of the Study, and the Results

Group	Number of Perforations
Group A	5 (38%)
Group B	2 (16%)
Group C	6 (46%)
Total	13

Nuboer (1951) in Holland strongly advocates immediate partial gastrectomy for perforated duodenal ulcer, and his views have mixed support. This series suggest that this operation is certainly not justified in all instances.

Effect of Dietary Regimes.—On leaving hospital, the members of this group were given dietary instructions by the Dietitian Service. These instructions were those usually in vogue in most clinics, including the avoidance of foods mechanically irritating, highly seasoned foods, condiments *et cetera*, the taking of limited quantities of fried foods, the sieving of vegetables and so on. Stress was laid on the regularity of intake of food and on the taking of extra milk between meals. All these men were in receipt of a pension, which is given in part to allow them to augment the ordinary diet with milk, eggs and cream.

Close questioning enabled us to classify these men into those who kept strictly to the dietary regimen, others who did so in part, and those who ignored the instructions and ate liberally. Table VIII shows that of those who kept to a strict regime, 57% were in the failure group and 31% in the satisfactory group. In the group of men who ate liberally, the percentages were 33% and 50%. These figures may be explained on the basis that those with recurring symptoms were forced to maintain a careful watch on what they ate, while those who ate liberally did so because they were free of symptoms. However, these findings suggest that in this series keeping to a strict dietary regime did not materially improve the prognosis.

Effect of Antacid Intake.—The particular type of antacid preparations taken by this group depended upon their own medical attendants. They included alkalis of all kinds,

TABLE VIII

Relationship between Ulcer Dietary Regime, and the Results

Regimen	Group A	Group B	Group C	Total
Strict	19 (31%)	11 (18%)	31 (51%)	61
Moderate	4 (27%)	2 (13%)	9 (60%)	15
None	12 (50%)	4 (17%)	8 (33%)	24

aluminium hydroxide gels, anticholinergic drugs such as " Banthine " *et cetera*. The group was divided into those who took antacids daily (often three or four times per day), those who took these preparations for symptomatic relief, and those who did not take them at all. The vast majority of the group were taking some antacid preparation.

Table IX indicates the finding that of those who took antacids daily, 60% were in the failure group and 27% in the satisfactory group.

TABLE IX

Relationship between Antacid Intake, and the Results

Intake of Alkalis	Group A	Group B	Group C	Total
Daily	16 (27%)	8 (13%)	36 (60%)	60
Symptomatic ..	16 (44%)	9 (25%)	11 (31%)	36
None	3 (75%)	—	1 (25%)	4

The likely interpretation for this is that those with symptoms were more likely to have taken antacids regularly than those without. However, these figures would appear to indicate in this group that the regular daily consumption of antacid preparations did not in any way improve the prognosis.

Effect of Smoking.—The series was divided into non-smokers, those who smoked moderately (up to two ounces of tobacco per week), heavy smokers (three to four ounces), and very heavy smokers (five to eight ounces). The findings are set out in Table X.

Of the non-smokers, the percentage in the satisfactory group was 45, in the failure group 35. For the moderate smokers, the respective percentages were 30 and 60. These percentage figures would suggest, therefore, that in this series the non-smokers had a better prognosis. However, it is of some interest that there

TABLE X

Relationship between Smoking, and the Results

Amount of Smoking	Group A	Group B	Group C	Total
None	9 (45%)	4 (20%)	7 (35%)	20
Moderate	16 (33%)	7 (12%)	25 (55%)	48
Heavy	6 (30%)	2 (10%)	12 (60%)	20
Very heavy ..	4 (33%)	4 (33%)	4 (33%)	12

were four men who smoked five to eight ounces of tobacco per week for over six years, and who were in the satisfactory group

Effect of Alcohol Intake.—As regards alcohol consumption, the series was divided into those who took no alcohol (strict teetotallers), those who had a moderate intake (two or three beers per day at the end of the day's work), and those who had a heavy intake.

Table XI shows that the percentage of teetotallers in the satisfactory group was 29 and in the failure group 52. Among those who drank alcohol moderately the respective percentages were 44 and 38. All the heavy drinkers were in the failure group.

TABLE XI

Relationship between Intake of Alcohol, and the Results

Intake of Alcohol	Group A	Group B	Group C	Total
None	14 (29%)	9 (19%)	25 (52%)	48
Moderate	21 (45%)	8 (17%)	18 (38%)	47
Heavy	—	—	5 (100%)	5

These percentage figures suggest that in this series abstinence from alcohol did not improve the prognosis and may have made it worse, that a moderate alcohol intake did not influence the prognosis one way or the other, and that heavy drinking is associated with a poor prognosis.

Effect of Emotional Stress.—In the discussions with this group of patients, problems of a domestic, financial, occupational and personal nature were freely discussed, and an estimate was made of the degree of emotional stress present. This varied over the years of observation, so that a simple classification was made into average stress, more than average stress and excessive stress.

Of those subjected to average stress, 44% were in the satisfactory group and 33% in the failure group. If the stress was above average, the percentages were 30 and 57 respectively, while if the stress was excessive 80% were in the failure group (Table XII).

TABLE XII

Relationship between Emotional Stress, and the Results

Degree of Stress	Group A	Group B	Group C	Total
Average	19 (44%)	10 (23%)	14 (33%)	43
More than average	15 (31%)	6 (12%)	28 (58%)	49
Excessive	1	1	6 (80%)	8

These observations suggest strongly that in this series emotional stress was an important prognostic factor; the greater the stress the poorer the outlook.

Effect of the Mental State.—Each patient in this series was interviewed by a psychiatrist on one or more occasions and a report was given of his mental state and outlook; 77% were classed as having no psychiatric disability, and of this percentage the proportions of those in the satisfactory and failure groups were much the same (Table XIII). The abnormal mental states were classed as anxiety states, personality defect and frank psychosis or psychoneurosis; 78% of patients in this category were found in the failure group.

TABLE XIII

Relationship between Psychiatric State, and the Results

Psychiatric State	Group A	Group B	Group C	Total
Psychiatric history nil	33 (43%)	14 (18%)	30 (39%)	77
Anxiety state ..	2	1	11	
Personality defect	0 } 2	1 } 3	3 } 18	23
Psychosis ..	0 } (9%)	1 } (13%)	4 } (78%)	

In this series, then, the mental state of the patient appeared to have an important prognostic bearing, and the presence of an anxiety state, personality defect or psychosis materially worsened the outlook.

We feel that too little attention is paid to the mental state of the patient in the assessment of results of treatment for duodenal ulceration,

whether medical or surgical. Indeed, mention of this factor is scarcely ever made in such reports appearing in the literature.

SUMMARY

A series of 105 male ex-servicemen suffering from uncomplicated duodenal ulceration were followed for periods varying from five to eight years. Five died from causes unrelated to their ulceration and were excluded from the study. The remainder were reviewed personally by us at intervals of six to twelve months.

The results of medical care were analysed according to certain criteria, and were classified into satisfactory, intermediate and failure groups, the numbers in each group being 35, 17 and 48 respectively.

These results were analysed in terms of duration of symptoms, incidence of hæmorrhage and perforation, dietary regimens, antacid therapy, tobacco and alcohol consumption, emotional stress and the patient's mental state. The effects on the prognosis of these factors were as follows: In this series, the longer the duration of symptoms the worse the outlook; hæmorrhage worsened the prognosis, but perforation did not necessarily do so; a strict dietary regimen and regular antacid intake did nothing to improve the results; tobacco affected the outlook adversely, but a moderate intake of alcohol did not; the greater the emotional stress, the poorer the results; a defective mental state materially worsened the prognosis.

We consider that in this series of patients, medical care did not affect the natural course of this disease in any appreciable way.

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ŒDEMA IN HEART FAILURE : LESSONS FROM THE BERI-BERI HEART

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THERE are two principal hypotheses concerning the formation of œdema in heart failure, the "backward failure" hypothesis and the "forward failure" hypothesis. According to the first, shown diagrammatically in Figure I, heart failure leads to a rise in venous pressure and in consequence to a rise in capillary pressure, the resulting transudation leading to œdema and

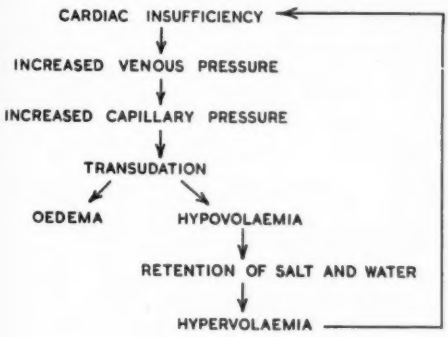

FIGURE I

A simplified version of "backward failure". The earlier stage of cardiac stimulation by hypervolæmia and the bone marrow and plasma protein changes have been omitted

at the same time causing a fall in the circulating blood volume which leads to retention of salt and water by the kidney.

According to the forward failure hypothesis, reduction of the cardiac output, assumed to be an inevitable consequence of failure of the pump, starves the tissues of blood; the symptoms and signs of heart failure are brought about simply by reduced blood flow (Mackenzie, 1913). This view, which was never completely formulated, has now been shown to be untenable (Harrison, 1935) and has been replaced by a new forward failure hypothesis which stems from the observations of Warren and Stead (1944).

These workers studied two patients with heart disease who were on the brink of œdema. After the administration of sodium chloride the body weight and plasma volume increased before the antecubital venous pressure rose. They concluded that the rise in venous pressure is not due to back pressure but depends on an increase in blood volume brought about by the failure of the kidney to excrete salt and water.

Subsequently it was shown that in congestive failure the filtration rate and the renal plasma

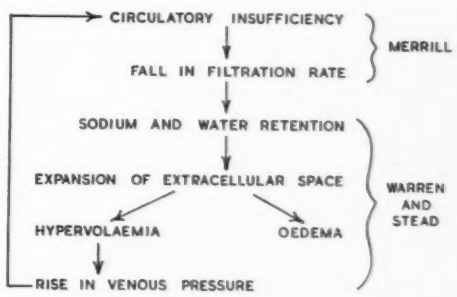

FIGURE II

"Forward failure", from Warren and Stead (1944) and Merrill (1948)

flow are much reduced and correlate well with the reduction of cardiac output but not with venous pressure. It was therefore argued that reduction in the quantity of sodium delivered to the tubules in the glomerular filtrate allows more complete reabsorption to occur; water is retained in proportion to sodium and the sequence observed by Warren and Stead comes into play (Merrill, 1946, 1948). This new concept of "forward failure" is shown in Figure II.

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It will be noted that according to the forward failure hypothesis the rise of venous and capillary pressure is not a direct consequence of failure of the pump, but occurs after the kidney has retained salt and water; on the backward failure hypothesis the rise of venous and capillary pressure is secondary to heart failure and the loss of fluid into the tissues is dependent on hydrostatic forces. The stimulus to fluid retention by the kidneys is reduction of the circulating blood volume.

The following observations, made incidentally in patients with beri-heri heart disease, throw some light on these hypotheses. The laboratory measurements have been made by generally accepted methods and will be described elsewhere.

Observation 1

A forty-one-year-old man entered the hospital complaining of weakness, anorexia and dyspnœa. Three weeks previously he had noticed swelling of his feet; this had disappeared spontaneously. There was a strong history of alcoholism. On examination he was restless and slightly jaundiced. The nail beds were cyanosed, the pulse rate was 140 per minute, and the blood pressure 110 millimetres of mercury, systolic, and 60, diastolic. Pistol shot sounds were audible over the arteries. The cervical venous pressure was three centimetres above the sternal angle. The liver was enlarged one finger's breadth; there was no œdema. The heart was not enlarged but the right ventricle was hyperdynamic. Clinically and radiologically the lungs were clear. The calves were tender and the knee and ankle jerks were absent. The blood pyruvate content was 4·0 milligrammes per 100 millilitres (normal <1·5 milligrammes) and the blood urea content was 79 milligrammes per 100 millilitres. After hæmodynamic studies the patient was given thiamine and recovered in a few days. Certain measurements before and a month after treatment are shown in Figure III.

In this patient the right auricular pressure was elevated and the blood pressure was low. There was no œdema, no significant change of weight with recovery and no diuresis.

It is therefore concluded that elevation of the venous pressure was a direct result of myocardial inadequacy and did not depend on fluid retention. The sequence found by Warren and Stead is therefore not applicable to heart failure in general. Moreover, it is at variance with the rise of venous pressure in the heart lung preparation when the heart fails (Patterson *et alii*, 1914) and in acute heart failure from other causes (for example, arrhythmias), in which raised venous pressure may occur within an hour and certainly before significant fluid retention can occur.

Secondly, although peripheral venous pressure was high, there was no œdema. It has been shown that in beri-beri the peripheral venous pressure rises more than the right auricular pressure. In the case of Lahey *et alii* (1953) with a very similar output to that in the present case the peripheral venous pressure was 31 centimetres of water and the gradient from peripheral vein to right auricle was 22 centimetres of water. It follows that in our own

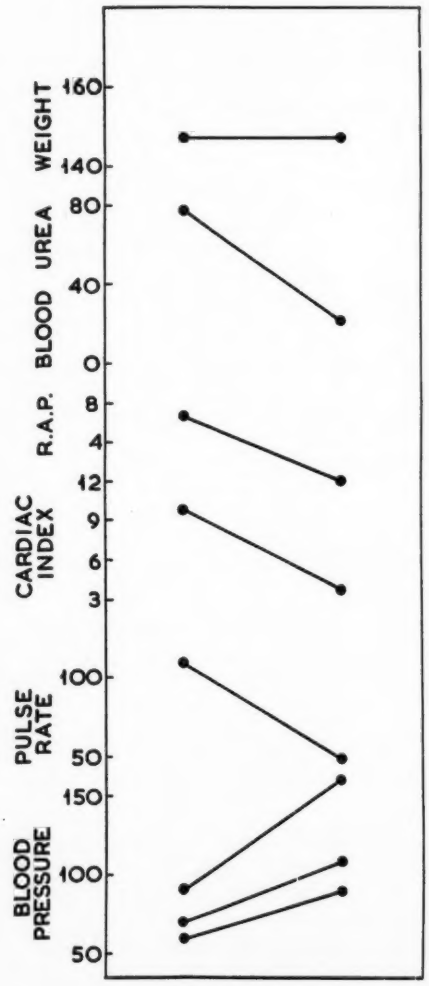

Figure III

Observation 1. Acute beri-beri. Findings before treatment and after recovery. Cardiac index in litres per minute, right auricular pressure (RAP) in millimetres of mercury, blood urea in milligrammes per 100 millilitres and weight in pounds. The forearm blood flow was 6·7 millilitres per 100 millilitres per minute twenty-four hours after treatment began and 2·4 millilitres per 100 millilitres per minute on recovery

case the capillary pressure must have been very high and of the order of the protein osmotic pressure of the plasma.

From the data of Landis and Gibbon (1933) a venous pressure of 30 centimetres of water would cause fluid to be filtered from the blood stream at the rate of 50 to 60 millilitres per minute. As œdema was not present in our case, either the fluid was returning to the blood stream at a similar rate (? via the lymphatics) or the filtering surface (number of capillaries) or permeability differed from the condition of their experiment. It would therefore appear that one cannot use results obtained with venous occlusion of a normal arm to explain the œdema of heart failure; for if Landis and Gibbon's observations held in our own case, then it is difficult to see why the patient did not develop œdema and why his blood volume did not fall to an unviable level. Unfortunately a technical error invalidated the actual measurement. But since the cardiac index was three times normal and the venous system was overfilled, it is hard to believe that the blood volume was much reduced.

It will be noted that in this patient the blood urea content was 79 milligrammes per 100 millilitres, and although they were not measured, we conclude that the renal plasma flow and glomerular filtration rate were greatly reduced. Measurements in five patients with moderate beri-beri heart disease have shown glomerular filtration rates of the order of 70 millilitres per minute and renal plasma flow of the order of 250 millilitres per minute. As œdema was not present in this patient, it would appear that the supply of salt and water to the body did not exceed the kidney's capacity to excrete them. It also seems unlikely that hypovolæmia, if it occurred at all, was the stimulus for renal vasoconstriction.

Finally the systolic, diastolic and mean blood pressures were all reduced and the nail beds were cyanosed, indicating vasoconstriction in the skin. The high cardiac output was inadequate to maintain the blood pressure or a normal flow to all the tissues of the body. Some tissues received more than their normal share of the cardiac output; the forearm blood flow twenty-four hours after thiamine was given was still three times normal. The combination of vasoconstriction in the skin and kidneys with a low blood pressure suggests that the stimulus to vasoconstriction in heart failure may lie on the arterial side of the circulation.

OBSERVATION 2

A forty-nine-year-old man complained of dyspnœa of two weeks' duration which had become more severe in the last thirty-six hours. He had been drinking heavily and had evidence of peripheral neuritis. He was restless and distressed. His respiration was rapid and deep; his face and the muscles generally were swollen, but there was no subcutaneous œdema anywhere. The pulse rate was 120, the blood pressure 90 millimetres of mercury, systolic, and 50, diastolic, and the antecubital venous pressure was 30 centimetres of water. The central venous column reached the lobe of the ear when he was in the semi-upright position. The heart was enlarged and hyperdynamic, gallop rhythm was present, and crepitations were present at the lung bases. The liver was palpable three fingers' breadth below the costal margin. Sweating of the trunk and neck was profuse, but the finger nails were cyanosed. The body weight and blood volume were increased 34 pounds and 3·04 litres respectively above the recovery values.

Within an hour of the administration of thiamine the blood pressure began to rise, the venous pressure to fall and the clinical condition to improve (Figure IV). The following day the blood pressure was 160 millimetres of mercury, systolic, and 80, diastolic; the pulses were bounding and the forearm blood flow was five times normal. The venous pressure had fallen to 23 centimetres of water, although there had been no diuresis. The blood urea content, which was 52 milligrammes per 100 millilitres on admission, rose to 118 milligrammes on the third day; the renal response to heart failure was still present.

In this patient restoration of the circulation in the first twenty-four hours was a pure hæmodynamic event without any overall change in the body fluids. Diuresis began on the third day when the venous pressure was still high and the cardiac output, although lower, was still not normal. Restoration of the circulation preceded renal excretion of salt and water.

This patient differed from the previous one in having considerable hypervolæmia and fluid retention; he resembled the other in having a low blood pressure and renal vasoconstriction.

The findings in both patients suggest that heart failure and recovery from heart failure are hæmodynamic events. Functional changes in the kidney with changes in body fluid volume are secondary. The venous pressure may rise before fluid is retained and may remain high while fluid is being lost. A high venous pressure, even in the presence of considerable hypervolæmia, does not lead inevitably to subcutaneous œdema. When fluid is available transudation occurs in those tissues where capillary surface and capillary pressure, transmitted from the arterioles, are greatest.

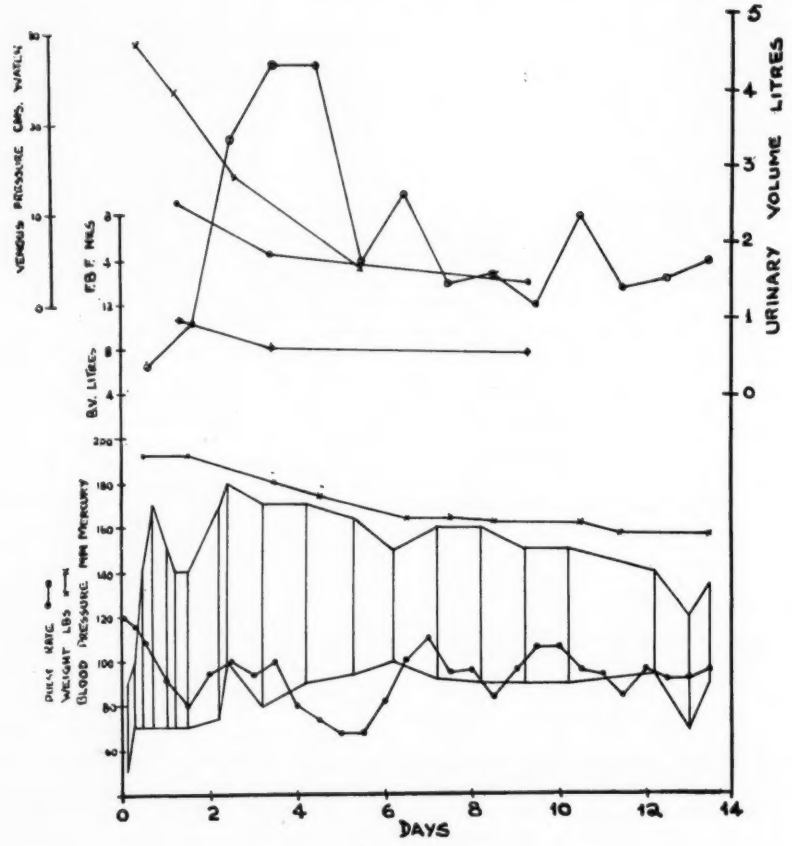

FIGURE IV

Observation 2. Acute pernicious beri-beri. Changes in pulse rate, blood pressure, weight, blood volume (BV), forearm blood flow (FBF), venous pressure and urinary volume. The venous pressure was measured with a saline manometer, reference point five centimetres behind the sternal angle. During the first two days when oliguria was present the blood urea rose from 52 to 118 milligrammes per 100 millilitres

Venous pressure ×——×
Forearm blood flow ●——●
Blood volume ◐——◐
Urinary volume ○——○

OBSERVATION 3

A forty-one-year-old alcoholic walked into the out-patient department complaining of swelling of the feet, anorexia, breathlessness, shivering and blueness of his hands of two weeks' duration. On examination he was cyanotic and the hands and feet were cold. The pulse rate was 120 per minute, the blood pressure 170 millimetres of mercury, systolic, and 90, diastolic, and pistol shot sounds were audible over the arteries. The cervical veins were full up to four centimetres above the sternal angle. The heart was slightly enlarged and gallop rhythm was present. The respiratory rate was 26 per minute. A few crepitations were heard at the lung bases. The liver was enlarged four fingers' breadth below the costal margin, the spleen was not palpable. There was pitting œdema of the legs up to the knees. Signs of peripheral neuritis were present. He was given digoxin and mersalyl and admitted to hospital next day. Some diuresis had occurred and the venous pressure had fallen overnight. The lungs were now dry and the systolic pressure lower, but the physical findings were otherwise unchanged. The fasting blood pyruvate content was 4·47 milligrammes per 100 millilitres. After treatment with thiamine he made a complete recovery.

Figure VA shows certain findings before treatment and on recovery. Figure VB shows the findings in a similar case reported by Lahey and others (1953). The two cases will be discussed together. The cardiac indices were approximately four and three times normal, respectively. Œdema was present and with recovery the losses of weight were 11 and 40 pounds.

The right auricular pressure was within normal limits in both patients and in Lahey's case did not change with recovery. In both patients the renal plasma flow and glomerular filtration rate were grossly reduced; in our patient the nail beds were deeply cyanosed. Despite enormous increase in muscle flow which accounted for a large part of the cardiac output, compensatory vasoconstriction in other parts of the vascular bed succeeded in keeping the blood pressure within normal limits.

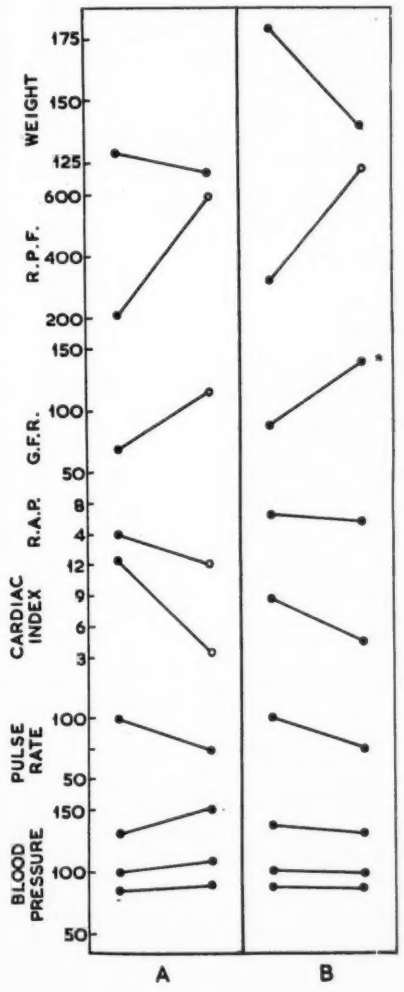

FIGURE V

Observation 3. Moderately severe beri-beri. (A) Findings before treatment and after recovery in Case III and (B) in the case of Lahey and others. The open circles are generally accepted normal figures; actual measurements were not made. Glomerular filtration rate (GFR) and renal plasma flow (RPF) in millilitres per minute. In our own case the forearm blood flow before treatment was 8·0 millilitres per 100 millilitres per minute after recovery 2·5 millilitres per 100 millilitres per minute. The corresponding blood volumes were 4·96 and 3·78 litres

In these two patients circulatory insufficiency was less marked than in the previous two. The blood pressure was normal and the central venous pressure was within normal limits.

Because the central venous pressure was normal renal vasoconstriction and, as Lahey found, failure of water and salt diuresis could not be the result of elevation of central venous pressure. As the renal venous pressure may have been higher than normal in all these cases, the possibility, though remote, remains that this may have been responsible for the behaviour of the kidney.

As the arterial pressure was normal and renal vasoconstriction was considerable, one must question the conclusion, suggested by the findings in the first two patients, that the fall in blood pressure was responsible for the changes in the kidney. In all three of our patients vasoconstriction was also present in the hand; actual measurement in Case III showed that the hand flow was 2·8 millilitres per 100 millilitres per minute and after prolonged reflex heating rose only to 3·0 millilitres. In the first two cases the most extreme vasoconstriction, with deep cyanosis in the hand, urea retention and oliguria, was unable to maintain the blood pressure. In the third case the blood urea content was 21 milligrammes per 100 millilitres and vasoconstriction was obviously less. It was nevertheless adequate to restore the blood pressure to normal. If low blood pressure is the stimulus for vasoconstriction in the kidney and the skin, then obviously its correction by vasoconstriction cannot be taken as evidence against low blood pressure as a stimulus. That the circulation was insufficient at rest in Case III was also suggested by the rise of right auricular pressure from four to 12 millimetres of mercury during gentle exercise. In both these cases regional vasoconstriction can be regarded as a sensitive index of circulatory insufficiency.

Thus far the findings are consistent with the view that failure of the cardiac output to maintain the blood pressure is closely related to the functional behaviour of the kidney. On the other hand the blood volume and venous pressure showed wide variations from case to case and did not correlate with renal function; because of the possibility that in the first case the blood volume was reduced we

shall now examine the relationship between plasma (or blood) volume and fluid retention in beri-beri.

OBSERVATION 4

Figure VI shows the relation between the loss of weight with recovery (a measure of fluid retention) and the fall in plasma volume in nine cases of cardio-vascular beri-beri of varying grades of severity. The data were obtained within the first few days of admission to hospital when in some instances bed rest alone had led to improvement. Only a few patients had been given thiamine.

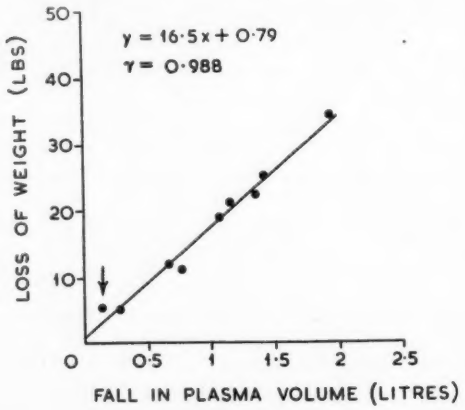

FIGURE VI

To show loss of weight and fall in plasma volume with recovery in beri-beri. Discussed in the text

There is a striking correlation between loss of weight and fall in plasma volume and the regression line, when extrapolated to zero fall in plasma volume, intercepts the weight axis at 0·79 pound, which is not significantly different from zero. It therefore appears that when fluid is retained in beri-beri it distributes itself in constant proportion between the intravascular and extravascular spaces; during recovery this proportionality is maintained. The short extrapolation to zero suggests that before fluid is retained the same proportionality is preserved; in other words that the plasma volume is normal. Is this conclusion justified?

If we assume for a moment that acute heart failure with a precipitous rise of venous and capillary pressure occurred (backward failure) so that 600 millilitres of plasma passed into the extravascular space and no change in weight occurred, when plotted on Figure VI the point would fall well outside the confidence limits of the regression line. Furthermore there is no evidence that the removal of 600 millilitres of blood by venesection will cause the renal plasma flow to fall to the levels observed in these cases of beri-beri.

Either the extrapolated regression line is invalid or "backward failure" does not hold. That the latter may be true is suggested by the following case.

A thirty-year-old man was brought to the hospital unconscious with acute pernicious beri-beri. The peripheral pulses could not be felt. The neck veins were congested, pulmonary œdema was present and there was no peripheral œdema. Severe acidosis was present (carbon dioxide combining power 6·3, chloride 102, sodium 152, and potassium 4·6 milliequivalents per litre). After administration of thiamine he recovered rapidly and within a few hours, when his acidosis was largely corrected, showed a typical hyperdynamic circulation with, as is usual, cyanosed finger nails. Twenty-four hours after his admission to hospital his cardiac output was 15·7 litres per minute (cardiac index, 8·4), his right auricular pressure was 14 millimetres of mercury, and his plasma volume and blood volume were 0·14 and 0·52 litre respectively above the recovery values. During the first twenty-four hours there was no change in weight and he passed, on a generous estimate, 250 millilitres of urine. Diuresis was established forty-eight hours after treatment. Again oliguria can be taken as evidence of severe renal vasoconstriction. He is represented in Figure VI by the point indicated by the arrow. When recovery had begun and when vasoconstriction was still severe the plasma volume and body weight were 0·14 litre and five pounds respectively above the recovery value. If hypovolæmia was the original stimulus to vasoconstriction, then some other stimulus must have been operative at the time the blood volume was measured. Furthermore at the time of his admission to hospital, when the pulses could not be felt, the peripheral veins were constricted and the skin was cold and cyanosed and the colour returned slowly after blanching. It was concluded that the capillary pressure in the skin was low and must have risen as the arterial pressure rose. With improvement hydrostatic forces in the capillaries must have favoured transudation rather than reabsorption. If, therefore, any change occurred at all in the blood volume in the first twenty-four hours, it should have been contraction rather than expansion.

We are left with the conclusion that in the most severe heart failure that can occur short of actual death, the capillary pressure is probably low and the blood volume is probably not reduced. There is no evidence that in beri-beri hypovolæmia is responsible for renal vasoconstriction and salt and water retention. We have been unable to demonstrate hypovolæmia in any stage of the disease.

To summarize—these cases of beri-beri have shown that the rise in right auricular pressure which accompanies heart failure is directly dependent on heart failure and not on prior fluid retention. Fluid retention and œdema are secondary phenomena which are the

resultant largely of the intake of salt and water and the kidneys' capacity to excrete them. The stimulus to renal vasoconstriction and sodium retention does not come from the venous side of the circulation ; rather does it appear to be related to the threatened or actual fall of blood pressure which results from failure of cardiac output. Lastly, in no stage of beri-beri does the blood volume appear to be reduced ; hypovolæmia must therefore be dismissed as the stimulus for salt and water retention. As hypovolæmia is an essential step in the backward failure hypothesis, the backward failure hypothesis must also be abandoned, at least in the beri-beri heart.

DISCUSSION

It is axiomatic that in low output failure as in high output failure the changes in the circulation to the kidney and in the excretion of salt and water must succeed failure of the pump. It is of interest therefore to consider what is the trigger stimulus for the kidney in low output failure—the rise in venous pressure, the fall in cardiac output, the fall in blood pressure or, assuming that the capillary pressure rises and transudation occurs, hypovolæmia ?

The Venous Pressure

If the venous pressure rise is the important stimulus, then it must act either reflexly from the right auricle or some peripheral organ, or directly on the kidney itself. Although the experiment of Fishman *et alii* (1950) on constrictive pericarditis has been interpreted by Katz (1954) as evidence in favour of an auriculo-renal reflex, in my view it remains unproven, and is not supported by the present observations. Furthermore the changes observed by Katz and his group were quantitatively inadequate to account for the gross changes in the renal circulation seen in beri-beri and other forms of heart failure. In support of this view Werko *et alii* (1954) have shown that renal vasoconstriction and reduced excretion of sodium can be demonstrated in patients with a low cardiac output from mitral stenosis when the right auricular pressure is normal. If it is assumed that in Werko's patients the renal venous pressure was also normal, then it follows that the renal changes in heart failure are also independent of the renal venous pressure. In this sense the earlier demonstration by Blake *et alii* (1949) and Selkurt *et alii* (1949) that elevation of renal venous pressure leads to sodium retention is irrelevant. Furthermore, in chronic experiments, partial ligation of the inferior *vena cava* leads to only temporary retention of sodium and water without appreciable change in renal plasma flow or glomerular filtration rate (Hwang *et alii*, 1950).

It can be concluded that elevation of venous pressure either in the right auricle or in the kidney is not the cause of the renal changes in heart failure, although renal venous hypertension may contribute to salt and water retention.

Nor is there any convincing evidence that elevation of the venous pressure in other parts of the body leads to renal vasoconstriction unless the blood volume and cardiac output are reduced at the same time by transudation. When the blood volume (and cardiac output) is restored by blood or saline, retention of salt and water ceases (Judson *et alii*, 1952 ; Fitzhugh *et alii*, 1953). That the changes produced by tourniquet compression are temporary and due to the fall in blood volume is also suggested by the absence of general œdema in chronic pathological obstruction of either of the great veins.

It could be argued that reduction of venous pressure stimulates renal vasoconstriction and sodium retention as the latter are seen in shock, hæmorrhage and after ingestion of hexamethonium. The truth appears to be that vasoconstriction is quite independent of the venous pressure.

Hypovolæmia

According to the backward failure hypothesis, hypovolæmia following transudation is the stimulus for salt and water retention. It is well known that following hæmorrhage renal vasoconstriction with salt and water retention occurs ; these changes tend to restore the blood volume and the blood pressure. Furthermore, the degree of vasoconstriction produced by severe bleeding or by prolonged tourniquet obstruction may be as great as occurs in heart failure (Wiggers, 1950 ; Wilkins *et alii*, 1953).

From earlier data Landis *et alii* (1946) calculated that a ten-centimetre rise of peripheral venous pressure will cause fluid to be filtered from the blood stream at a rate of 25 to 30 millilitres per minute, which they believed is of sufficient order to cause hypovolæmia and compensatory vasoconstriction in the kidney with salt and water retention.

If this sequence occurred in acute heart failure, it should be possible to demonstrate a marked reduction of blood volume and by inference a rise in capillary pressure. To date this has not been done ; because he thought that capillary pressure must fall, Starling

(1896, 1897) originally rejected the backward failure hypothesis. Actual measurements of the blood volume itself after coronary thrombosis have shown it to be within the normal range (Fishberg *et alii*, 1934), although whether this is relevant is doubtful as the left ventricle usually fails rather than the right. Measurements during acute failure from an arrhythmia may prove more helpful. In practice oliguria may occur in both conditions. If it is assumed for a moment that hypovolæmia does occur, there is still the difficulty of explaining why, when it is corrected, the kidney continues to retain salt and water. Starling (1909) and subsequent workers have therefore introduced the concept of "effective" blood volume, implying that blood pooled on the venous side of the circulation is "ineffective". By inference "effective" blood could be on the arterial side and the stimulus to the kidney could be arterial under-filling. But if the immediate consequence of failure of the pump is failure of output then arterial underfilling must occur irrespective of whether capillary filtration increases or not. In this view transudation from the capillaries is not an essential antecedent for salt and water retention, but when it occurs is merely a contributing cause.

The Cardiac Output and the Arterial Blood Pressure

It is generally accepted that as the competence of the heart lessens, the venous pressure rises and the cardiac output falls; when vasoconstrictive reactions are no longer adequate the blood pressure falls. As the rise in venous pressure seems inadequate to account for the change in renal function it is pertinent to examine the possibility, suggested by Borst (1938, 1948) and by Merrill, that the renal changes depend on the fall in cardiac output.

It has been noted already that after hæmorrhage and in tourniquet compression not only does the blood volume fall but the cardiac output falls also. If compensatory vasoconstriction is blocked at the same time the arterial pressure also falls. In both types of experiment retention of salt and water and renal vasoconstriction of the order seen in heart failure occur. Lesser variations are seen in changing from the supine to the erect position and these are accentuated by ganglionic blocking agents, both in normal and in hypertensive subjects.

In dogs, both Levy and Berne (1951) and Post (1951) found that reduction of cardiac output by constriction of the pulmonary artery and by pericardial tamponade respectively caused retention of salt and water followed by renal vasoconstriction which could not be explained by the rise in venous pressure and was unlikely to have been due to changes in blood volume. As in man, the blood pressure tended to be maintained by a rise in peripheral resistance.

In man the sensitivity of renal vasoconstriction is such that reduction of the cardiac output by a litre a minute reduces renal plasma flow by about 25% (Fitzhugh *et alii*, 1953). From the beginning the kidney shares in the vasoconstrictor reflex when the arterial pressure is threatened by a fall in cardiac output. If the kidney did not behave in this way and the blood pressure were maintained by extrarenal mechanisms, the kidney would receive no signal.

In low output failure, unless extreme, vasoconstriction usually maintains the blood pressure. In the high output failure of beriberi vasoconstriction cannot compensate for the arteriolar dilatation of the muscles. The diastolic pressure usually falls and rises with recovery (Figure VII).

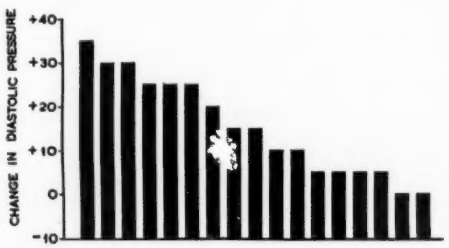

FIGURE VII

To show the change in diastolic pressure with recovery in 17 cases of cardiac beri-beri. The diastolic (and mean) pressure usually rises (shown as +). The diastolic pressure taken was that prevailing after the hypertensive phase of recovery had passed

The occurrence in these circumstances of severe renal vasoconstriction, even when the central venous pressure is within normal limits, and the dissociation of this change from changes in the blood volume may be taken as further evidence that arterial underfilling is the prime stimulus to renal vasoconstriction. That it will cause salt and water retention when the renal arterial pressure is reduced without reduction in blood flow has been demonstrated previously (Selkurt, Hall and Spencer, 1949; Selkurt, 1951). But it is only when the circulation as a whole is failing and blood begins to be diverted from the kidneys that vasoconstriction with ultimate

reduction of filtration rate complicates the picture of increased tubular reabsorption.

At present it is impossible to decide the tolerance of the renal vasculature to pressure changes. Nor is there any explanation why sodium and water retention frequently precede and exceed in degree changes that can be accounted for by hæmodynamic alterations. Indeed it is possible that the electrolyte and renal vascular behaviour are independent for in the experiments of Selkurt (1951) salt and water retention occurred when renal vasodilatation was present and natriuresis when vasoconstriction was present. This latter was also the case in the fistula experiments of Epstein *et alii* (1953). Without explaining the mechanisms, both reactions could be accounted for on teleological grounds, salt and water retention being aimed at maintenance of the blood volume and renal vasoconstriction at maintenance of the blood pressure and filtration rate and elimination of waste products.

The mechanism of renal vasoconstriction is not yet certain. It is presumed to be non-neurogenic and independent of the carotid sinus, for it continues despite spinal anæsthesia (Mokotoff *et alii*, 1949). Although humoral factors have yet to be ruled out it is possible that it represents an autonomous renal reaction to change in perfusion pressure. Yet there is evidence that vasoconstriction in the hand may be neurogenic for it appears to be abolished by reflex vasodilatation in beri-beri and by hexamethonium in congestive heart failure (Burch, 1954).

Similarly the mechanism of salt and water retention is unproved. Selkurt's work suggests that it may be due to hæmodynamic factors affecting the tubules, though there is still a tendency to attribute it to adrenal cortical hormones. However, it has recently been shown that patients with Addison's disease respond to postural changes, congestion of limbs and hypotonic expansion of the extracellular fluid volume in the same way as normal subjects (Rosenbaum *et alii*, 1952). The following case in which cardiac failure, probably due to beri-beri, complicated Addison's disease, shows that the adrenal cortex cannot play more than a subsidiary role.

OBSERVATION 5

A sixty-one-year-old woman was admitted to the Royal Prince Alfred Hospital on May 18, 1954. She had complained of pigmentation of the skin, loss of weight and weakness for five months. Her appetite was poor and œdema of the ankles had appeared four months before her admission. Three weeks before admission she became dyspnœic and swelling of the legs became more marked.

On admission she showed Addisonian pigmentation, œdema, enlargement of the heart and gallop rhythm. Her blood pressure was 145 millimetres of mercury, systolic, and 90, diastolic. Her electrocardiogram was abnormal. There was no change in her eosinophile count after 40 units of ACTH, and skin biopsy was consistent with Addison's disease. X-ray examination revealed chronic tuberculosis of the lungs, vertebræ and adrenal glands. The serum sodium content was 127 milliequivalents, potassium 7·0 milliequivalents and chloride 85 milliequivalents per litre. Total serum protein content was 4·7 grammes and blood urea 31 milligrammes per 100 millilitres. Following therapy with digoxin, mersalyl and thiamine the heart failure subsided and the heart size decreased. The patient was then treated with DOCA and salt and made an excellent clinical and biochemical recovery from adrenal disease although still immobilized by Pott's disease of the spine.

Although her hypoproteinæmia doubtless contributed to œdema formation, her recovery from heart failure occurred before the serum protein levels altered significantly. The occurrence of heart failure with œdema in full-blown Addison's disease makes it unlikely that the adrenal cortex plays an important part in the œdema of heart failure.

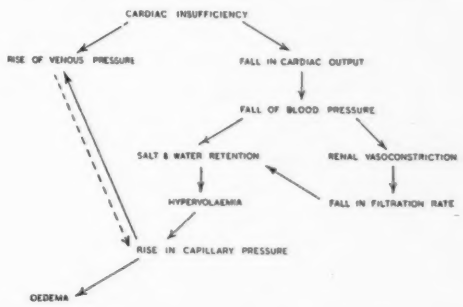

FIGURE VIII

A simplified representation of the sequence of events in heart failure; œdema is chiefly a by-product of the renal reaction to the fall of blood pressure brought about by failure of output

The evidence both from these observations on the beri-beri heart and from the work of others is therefore consistent with the view that the changes in renal function in congestive heart failure are due principally to a reduction in cardiac output with consequent underfilling of the arterial tree. It implies that the kidney plays an important part in acute regulation of the blood pressure. Evidence is also growing that it plays a part in chronic blood pressure regulation (Wilson, 1953). The sequence of events is shown in Figure VIII.

On this view both high output and low output failure lead to œdema in much the

same way. Once the heart is unable to maintain the ideal volume flow to the tissues at the ideal pressure for the individual, blood begins to accumulate on the venous side of the circulation and the arterial tree becomes underfilled. This evokes reflex vasoconstriction in the kidney and other parts of the circulation and probably leads directly to salt and water retention. During muscular exercise the same adaptative reaction is seen in normal individuals and it is not illogical to assume that the reaction seen in one form of stress is similar to that seen in another. In exercise, dilatation of the muscular arterioles increases the run off from the arterial tree which is theoretically underfilled. The arterio-venous oxygen difference in non-exercising limbs increases. The rise in arterial pressure which occurs in exercise may be converted into a fall by doses of hexamethonium which reduce only slightly the arterial pressure in the upright posture (Ronnov-Jessen, 1953; Fowler and Guz, 1954). Under these conditions the adaptative reaction by the kidney occurs at a time when the heart has not failed in the usual sense. When the cardiac competence is diminished it can be provoked more easily (Kattus *et alii*, 1949) and is a much more direct and sensitive response than that depending on preliminary transudation through the capillaries.

Œdema can be looked upon as a byproduct of the renal response to heart failure. When the cardiac output falls below the optimum, the fall of blood pressure leads directly to salt and water retention and to renal vasoconstriction. Instead of remaining in the blood stream and, by raising the venous pressure, stimulating the heart to refill the arterial tree, most of the fluid passes into the tissues. This is the pathological expression of a mechanism which the kidney has evolved to maintain the cardiac output in times of stress.

The present observations, while not disproving, do not support the suggestion of Landis *et alii* (1946) that repeated rises of venous pressure brought about by daily activity may, by "back pressure", increase capillary transudation. The crux of the matter, as Starling recognized, is the capacity of the venous system, affected as it undoubtedly is by changes in venous tone. When the venous capacity is exceeded, capillary pressure must rise; that this can occur without prior fluid retention remains unproven. The present findings suggest that such a mechanism is of secondary importance.

Summary and Conclusions

The hæmodynamic findings in a few selected patients with high output failure due to beri-beri have been described. They show that heart failure is primarily a hæmodynamic event which is independent of changes in fluid balance. As in low output failure, the hæmodynamic situation is independent of the extent of œdema as judged by the loss of weight with recovery. The evidence is compatible with the view that failure of output with consequent underfilling of the arterial tree leads directly to vasoconstriction and salt and water retention by the kidney. There is no evidence that the behaviour of the kidney is secondary to changes on the venous side of the circulation. The present observations do not exclude the possibility that changes on the arterial side lead indirectly to renal functional changes. Nor is is implied that other modifying effects do not occur. The evidence from Addison's disease is against the view that adrenal cortical hormones play an essential part in the renal response to heart failure.

It is suggested that the renal response to circulatory insufficiency is a fundamental homœostatic mechanism and that œdema is a byproduct of its activity. The extent of œdema will depend on the severity and duration of the circulatory insufficiency and the supply of salt and water to the body. Its distribution, which in beri-beri differs from that in low output failure, must depend on local physical forces.

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THE AVAILABILITY OF IRON IN MEAT : SOME EXPERIMENTS WITH RADIOACTIVE IRON[1]

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ANIMAL MEAT is one of the richest sources of iron in the human diet, and in some countries the iron of meat represents a major portion of the total dietary iron. It is not known, however, whether the protein-bound iron of meat can be used by man. There is evidence that before absorption iron must be in the ionized state and in the reduced or ferrous form (Moore *et alii*, 1944). The iron in animal tissues is bound to organic protein material, from which it must be split either by cooking or by digestive enzymes. Some animals on a purely carnivorous diet are apparently able to utilize animal tissue iron, because the total iron in the body of dogs is comparable to that of man on a mixed diet. On the other hand, it has been questioned both on clinical and on chemical grounds whether meat is of any value to the iron-deficient patient.

The work described in this paper was undertaken to ascertain the value of meat as a source of iron in man. Briefly, a tracer dose of radioactive iron (Fe^{59}) was injected into a sheep, and twenty-one days later the tissues of the animal were prepared as food. Various parts of the food were fed to volunteers, and the absorption of iron was determined by measuring the radioactive iron in the blood of the volunteers.

When radioactive iron is injected intravenously or absorbed from the gastro-intestinal tract, it rapidly disappears from the blood serum and is deposited in the body stores, principally in the liver and spleen. Gradually it becomes incorporated into the developing red cells, the maximum amount appearing as hæmoglobin in fifteen to twenty days (Dubach *et alii*, 1946). The percentage of the injected dose which appears in the blood cells is dependent on the functional capacity of the hæmatopoietic tissue and also on the relative amounts of iron already present in the body as hæmoglobin iron and as storage iron in the form of hæmosiderin and ferritin. Under normal circumstances 75% to 80% appears in the hæmoglobin, but the percentage is much lower in patients suffering from hæmochromatosis, in which the iron stores are greatly increased.

The experiments to be described are based on these physiological considerations. Admittedly absorption of radioactive iron from labelled food could have been detected by determining the difference between the amount ingested and the amount excreted in the fæces. This method would have been independent of the marrow activity and of the extent of the iron stores of the subjects ; but measurement of the amount of absorbed iron appearing in the blood was preferred for a number of reasons.

There is little interchange between the dynamic iron of the body—red cells and stores—and the iron of myohæmoglobin and of the tissue hæmatin enzymes. Apparently the metabolism of this latter iron is very slow. As a consequence, injected radioactive iron does not appear in the non-storage organs of the body except as hæmoglobin.

METHODS AND MATERIALS

Radioactive Iron

Radioactive iron was obtained from the Atomic Energy Research Establishment, Harwell, through the Commonwealth X-Ray and Radium Laboratory, Melbourne. The iron content of the isotope solution was 0·84 microgramme per millilitre, and the activity per gramme of solution was 16·7 microcuries as Fe^{59} with a half-life of 45·1 days.

Sheep

A sheep weighing approximately 80 pounds was supplied by the McMaster Laboratory, Commonwealth Scientific and Industrial

[1] Received on August 16, 1955.

[2] This work was undertaken during tenure of a Fellowship of the National Health and Medical Research Council.

Research Organization, Sydney. It had lambed about fourteen weeks previously. The isotopic iron solution was injected intravenously in a divided dose on two successive days, so that a total of 50 microcuries was injected. After twenty-one days it was assumed that a maximum amount of radioactive iron was present in the blood, and the sheep was slaughtered by exsanguination from the vessels of the neck. The blood was collected into large beakers containing sodium citrate solution, a total of 2·25 litres of blood being obtained. The liver, kidneys and one leg were prepared for eating, and aliquots of these tissues and of the blood were kept for radio-assay.

Preparation of Food

With the addition of suitable flavourings and cereals, measured amounts of the blood were placed in large sausage skins. These were boiled in water for approximately thirty minutes, until the red colour changed to black. They were allowed to cool and the sausage skins were removed before the contents were eaten. Two portions of the liver were baked separately with water for approximately ten minutes and the kidneys were similarly treated. The leg of mutton was roasted in the conventional manner for approximately two hours.

Normal Human Volunteers

Four males and three females volunteered for the experiment. All had acted as blood donors six days prior to the experiment, and between 400 and 500 millilitres of blood had been removed from each subject. Hahn *et alii* (1943) have shown that the amount of iron absorbed from a test dose is increased after blood is removed, but that maximum absorption does not occur for about seven days.

Patients with Hæmochromatosis

The four patients with hæmochromatosis who took part in this experiment were all being treated for the condition. Five hundred millilitres of blood had been removed from each on many occasions, usually, but not invariably, at weekly intervals. Blood had been removed from each within ten days of ingestion of the sheep's tissues. Hæmochromatosis is characterized by excessive absorption of iron from the gastro-intestinal canal (Granick, 1949), and patients with this disorder were selected for the experiment because it was thought that absorption of minimal amounts of iron from meat would be more readily demonstrable in them than in normal subjects.

Ingestion of Food

Five subjects ingested the sheep's blood. Originally it was intended that only four should be involved, but one subject with hæmochromatosis became nauseated after eating only one-quarter of his share. The remainder was eaten by a fifth volunteer. All volunteers ingested the food at two meals on successive days. The liver, kidneys and muscle tissue were eaten at a single meal.

Preparation of Samples

Fifty millilitres of blood were obtained from each subject twenty-one days after the animal tissue had been ingested. These samples and the aliquots of the sheep's tissues were digested in Kjeldahl flasks with sulphuric and perchloric acids. To each sample, 10 milligrammes of carrier iron were added in the form of ferrous ammonium sulphate, and the total iron was precipitated quantitatively by neutralization of the solution with concentrated ammonia. The precipitate was centrifuged, the supernatant was removed and the precipitate was redissolved in 3M sulphuric acid. The volume was finally made up to 10 millilitres with 3M sulphuric acid.

Assay of Radioactivity

All measurements for radioactivity were performed in the Department of Physics, University of Sydney. A double-walled liquid Geiger counter was used (type M6, manufactured by 20th Century Electronics). This was mounted in a lead castle of one-inch wall thickness in order to reduce the background counting rate. The pulses from the counter were fed to a scaling circuit and registered in the conventional manner. The background counting rate, determined with the counter filled with distilled water, was 13 per minute. All recorded rates have been corrected by subtraction of this background.

Expression of Results

It was not possible to assess the distribution of absorbed iron between the blood and the tissues of the volunteers, and for practical purposes that in the tissues was ignored. The hæmoglobin value of the sheep's blood was found to be 10 grammes per 100 millilitres, and the iron content was calculated from this value (Hüfner, 1894). The iron content of the tissues was obtained from tables compiled by Marston and Dawbarn (1944). With this information and the results of the radio assays, it was possible to calculate both the percentage and the amount of iron in the meat of the sheep

which was absorbed and appeared in the blood of the volunteers. The methods used for calculation were as follows:

1. Blood volume of volunteers = (Weight in kilograms) × 78 millilitres[1].
2. Radioactivity of total blood volume = (Blood volume) × (radioactivity in one millilitre of blood of volunteer).
3. Absorbed iron appearing in blood of volunteer:

$$\text{Percentage} = \frac{\text{Radioactivity of volunteer's blood}}{\text{Total radioactivity of food ingested}} \times 100.$$

$$\text{Amount} = \frac{(\text{Iron content of food ingested}) \times (\text{Percentage absorbed})}{100}$$

All the radioassay values were corrected to a common date with the aid of a decay table before being used in the calculations. This was necessary because the assays were not all performed on the one day.

RESULTS

The results obtained for the subjects who ingested the sheep's blood are shown in Table I.

TABLE I

Absorption of Hæmoglobin Iron from Food

Subject	Amount of Sheep's Blood Ingested (Millilitres)	Iron Ingested (Milligrammes)	Absorbed Iron in Blood	
			Milligrammes	Percentage
Normal male ..	500	167	4·2	2·5
Normal female ..	500	167	6·2	3·7
Hæmochromatotic (male)	375	125	7·4	5·9
Hæmochromatotic (male)	500	167	5·9	3·5
Hæmochromatotic (male)	125	42	3·9	9·3

In the calculations for this and Table II, no attention has been paid to the small amount of iron in the plasma. It will be seen that the normal male ingested 500 millilitres of blood containing 167 milligrammes of iron, and that 2·5% or 4·2 milligrammes was absorbed and appeared in the blood. Figures for the normal female with the same intake were 3·7% and 6·2 milligrammes.

The three patients with hæmochromatosis all demonstrated absorption and utilization of hæmoglobin iron. The percentage was greater than with the normal male, but there was some variation between the subjects. Their tissue iron stores were probably increased because of the disease from which they were suffering. At least 15 grammes of iron had been removed from each subject as part of therapy (Davis and Arrowsmith, 1950), but it is not unlikely that the iron stores of the subjects were still large. The values shown in Table I probably represent a much smaller percentage of the total iron entering the body than with normal volunteers, because proportionately more absorbed iron would be found in the storage organs of the hæmochromatotic patients. It should be noted that the total iron intake of the three subjects with hæmochromatosis varied. The relationship between dosage and absorption with different iron compounds is not known, but it is perhaps significant that the last subject showed a high percentage absorption with relatively low absolute absorption from a small intake.

TABLE II

Absorption of Iron from Liver and Kidneys

Subject	Amount of Food Ingested (Grammes)	Iron Ingested (Milligrammes)	Absorbed Iron in Blood	
			Milligrammes	Percentage
Normal male ..	Liver (370)	51·0	4·1	8·0
Hæmochromatotic (male)	Liver (290)	40·0	8·3	20·8
Normal female ..	Kidneys (88)	11·6	2·4	20·7

The results obtained with the volunteers who ingested liver and kidney tissue are shown in Table II. The subject with hæmochromatosis absorbed a greater amount of iron from the liver than did the normal male. Twice the amount of absorbed iron was found in his blood, although the total amount ingested was less. It will also be seen that in all instances the amount of iron ingested was less than was ingested in the form of hæmoglobin by the first group of volunteers. However, it is apparent that the normal subjects and the patient with hæmochromatosis all absorbed proportionately more of the tissue iron. For example, a hæmochromatotic subject assimilated into his blood 8·3 milligrammes from a total of 40 milligrammes of iron in ingested liver tissue, whereas a similar subject ingested 167 milligrammes of hæmoglobin iron and showed only 5·9 milligrammes in his blood.

No radioactivity could be detected in the blood of the three subjects who each ingested 325 grammes of cooked muscle tissue from the

[1] The figure used for calculating the blood volume is that given by Gibson and Evans (1937).

sheep. Only insignificant amounts of radioactive iron were found in the aliquot of muscle tissue, presumably because the tissues were almost completely exsanguinated at the time of slaughtering.

DISCUSSION

The experiments reported were designed to investigate the availability of iron from cooked meat. The iron present in the hæmoglobin and that in the form of storage compounds in various organs was tagged by injected radioactive iron, and its availability directly measured. Other iron compounds in tissues could not be traced in this way; but it is possible that the availability of their iron is not unlike that of hæmoglobin iron, because their chemical structure is similar to that of hæmoglobin.

The results show that some hæmoglobin iron is available for absorption and utilization by man. They also suggest that this iron is not as readily available as is that in liver and kidney. In these organs iron is present in several different forms. Some exists in the hæmoglobin of the contained blood, of which a proportion is available for absorption. The bulk is in the form of hæmosiderin and ferritin, the chemical compounds which contain the iron held in reserve for hæmatopoiesis. The relationship between hæmosiderin and ferritin is still not clear, but it is probable that the two compounds are allied (Gabrio *et alii*, 1953). Iron is loosely bound to the protein in ferritin (Granick, 1946). This possibly accounts for the relative ease with which iron is liberated from liver and kidney tissue and absorbed into the body.

The tissues also contain iron in the form of various enzymes such as cytochrome, catalase and peroxidase. These all have an iron porphyrin compound in their structure, but there is no direct information available as to whether the iron can be released in the digestive tract. As has already been mentioned, injected radioactive iron does not enter into these hæmatin enzymes. However, they vary in their resistance to chemical denaturation, and it would seem likely in view of the results obtained with hæmoglobin that some of the iron in the hæmatin enzymes would be available for absorption.

Muscle tissue is undoubtedly the most common form of meat in the human diet. In addition to the iron of the hæmoglobin in the contained blood, muscles contain hæmatin enzymes and myohæmoglobin, and these compounds cannot be traced with radioactive iron. Myohæmoglobin has a molecular weight which is approximately one-quarter of that of blood hæmoglobin; but the chemistry of the two compounds is similar in many respects. It is probable that the iron of myohæmoglobin can be released in the same manner as the iron of blood hæmoglobin, but there is no direct evidence on this point.

The finding that iron can be released from hæmoglobin in food and subsequently be made available for absorption by man raises the question of the mechanism by which this is achieved. It seems unlikely that the hæmatin molecule can be absorbed as such, and it is probable that the iron must first be released in the ionized state. The process of cooking may denature the proteins and at the same time liberate some of the bound iron. Chemical factors in the stomach and intestines may also play a part, and bacterial action in the intestines is possibly involved. It is significant that large amounts of free porphyrins have been found in the fæces after intestinal hæmorrhage (Lemberg, 1955), which indicates that hæmoglobin is certainly degraded in the intestines.

SUMMARY

Experiments have been undertaken to investigate the availability to man of iron in animal meat. A tracer dose of radioactive iron (Fe^{59}) was injected into a sheep, and twenty-one days later blood, liver, kidneys and muscle tissue were obtained from the slaughtered animal. These materials were eaten by volunteers and the level of radioactivity in their blood was subsequently determined. It was found that hæmoglobin iron was absorbed, but to a lesser degree than was the iron in liver and kidney tissue. No direct evidence was obtained as to the availability of the iron of myohæmoglobin and of the hæmatin enzymes of the tissues; but it seems likely that these compounds may also release some iron in the intestinal canal for absorption. The mechanism by which iron is released from tissues is not known and requires further investigation.

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STORAGE IRON IN THE FŒTUS[1]

I. KALDOR[2]

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ABOUT 90% of the total amount of iron in the human body can be accounted for in two physiologically and chemically distinct fractions. The larger fraction consists of the iron in the hæmoglobin of the circulating red blood cells. The smaller fraction consists of iron in the form of storage compounds. These comprise ferritin and hæmosiderin and possibly a small quantity of other compounds whose chemical nature is yet unknown (Drabkin, 1951). The storage compounds are located chiefly in the liver and spleen, and from them a considerable amount of iron can be mobilized at short notice for hæmoglobin synthesis by the bone marrow (Shoden, Gabrio and Finch, 1953).

The body of the new-born infant contains a relatively larger amount of iron than the body of an adult (Widdowson and Spray, 1951), mainly because the concentration of hæmoglobin is about 50% higher in the blood of the new-born (Walsh, Arnold, Lancaster, Coote and Cotter, 1953). Serum iron value and percentage saturation of the iron-binding capacity of serum are also high in the new-born (Laurell, 1947), indicating the presence of relatively large iron stores.

In the work to be described in this paper, the size of iron stores was measured in a number of infants who were born at different stages of the third trimester of pregnancy and did not survive. It was found that while full-term infants had ample iron stores, the iron stores of those born prematurely were small in relation to body weight.

MATERIAL AND METHOD

Measurements were made on livers only, because the liver is the most important organ for iron storage, especially in man, in whom the spleen is of relatively small size. The specimens were received from the Royal Hospital for Women, Paddington, New South Wales, and from St. Margaret's Hospital, Darlinghurst, New South Wales. They had been obtained at autopsy performed on still-born infants or on infants who died shortly after delivery.

[1] Received on September 19, 1955.

[2] This work was undertaken during tenure of a Fellowship of the National Health and Medical Research Council.

Specimens were obtained from 16 infants whose clinical history and autopsy had indicated no pathological condition which could have affected the iron content of the liver. The clinically estimated fœtal ages of the 16 infants ranged from thirty to forty-three weeks, their body weights from 2·5 to 8·5 pounds.

The storage iron content of the livers was measured by a method developed in this laboratory (Kaldor, 1954), in which all the non-hæmoglobin iron is extracted from the tissues. At the same time not more than 5% of the hæmoglobin iron present in the tissue is extracted.

RESULTS

Because the clinically estimated age of the fœtus is generally considered unreliable, the results have been presented by relating the iron content of the liver to the weight of the fœtus. However, it is realized that the weight of the fœtus is not necessarily proportional to age.

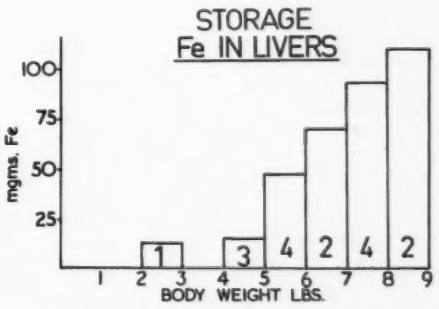

FIGURE I

The total iron content of the livers plotted against body weights. The height of each column shows the mean total storage iron content of the livers within the respective class. The figures inside the columns represent the number of fœtuses within the class

Figure I shows the total iron content of the livers plotted against body weights. The graph shows that the storage iron content of the livers of infants weighing less than five pounds is small, and that the iron content rises rapidly as the fœtus gains weight. There is nearly ten times as much iron in the liver of

infants weighing eight to nine pounds as in those weighing less than five pounds at birth. In other words, the storage iron in the liver increases about three times faster than the body weight.

Figure II shows the concentration of iron in the liver plotted against body weight. It is clear that the large amounts of storage iron in the livers of large new-born infants are due, not only to increased liver weight, but also to a higher concentration of iron.

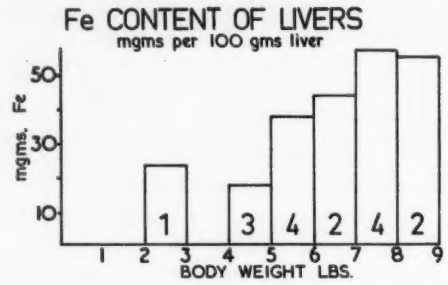

FIGURE II

The concentration of iron in the livers plotted against body weight. The height of the columns represents the mean for the respective class. The numbers within the columns show the number of fœtuses in the class

DISCUSSION

The total storage iron in a man of 70 kilograms body weight is approximately 750 milligrammes (Drabkin, 1951), or about 11 milligrammes of storage iron per kilogram of body weight. The storage iron content of the liver alone in infants weighing over six pounds at birth as reported in this paper is 27 milligrammes per kilogram. This is nearly three times the total adult value, which includes the storage iron in the spleen, bone marrow and kidneys in addition to that in the liver. If the total storage iron of the infants had been measured, instead of that in the livers only, the ratio of storage iron to body weight would have been more than three times the adult value.

The iron reserves of prematurely born infants, on the other hand, are low in relation to body weight compared with full-term infants. The liver storage iron content was found to be 14 milligrammes per kilogram of body weight for infants weighing less than six pounds at birth. Widdowson and Spray (1951) measured the total iron content (hæmoglobin iron and storage iron) of fœtuses of different ages, and found that it was proportional to body weight. The present findings suggest, therefore, that up to the third trimester a relatively larger proportion of the iron transferred from mother to fœtus is utilized for hæmoglobin synthesis, but relatively more of the iron transferred during the third trimester is used for building up reserves.

The lack of adequate reserves is probably a contributory cause of the iron deficiency anæmia which becomes manifest during the early months of extrauterine life in prematurely born infants. The dietary iron intake is low during the early months because of the low iron content of milk. The full-term infant is able to compensate for the deficiency of iron in the diet by calling upon its own large reserves, but the prematurely born infant becomes anæmic because it has inadequate reserves.

SUMMARY

Storage iron was measured in the livers of 16 infants who were born at various stages of the third trimester of pregnancy, and who were either stillborn or died shortly after birth. In prematurely born infants the storage iron of the liver increased both in amount and in concentration as the body weight increased. The mature or full-term infant was found to have a much greater amount of iron in proportion to body weight than has the adult. The bearing of these findings on the anæmia of prematurely born infants is indicated.

ACKNOWLEDGEMENTS

The writer wishes to acknowledge the assistance of Dr. J. L. Holme, of the Royal Hospital for Women, and of Dr. Margaret Collins, of Saint Margaret's Hospital, in obtaining liver specimens.

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COMPLEMENT IN ACQUIRED HÆMOLYTIC ANÆMIA[1]

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BECAUSE of the paramount role of complement in immune hæmolysis *in vitro* and its disappearance as the reaction occurs, it might be expected that in patients suffering from hæmolytic anæmia due to an immune mechanism, the occurrence and severity of hæmolysis would be reflected by a depression of the blood level of complement.

In 1909 von Stejskal (see Dameshek and Schwartz, 1940) reported a low serum complement level in acquired hæmolytic anæmia, and in 1911 Widal (see Tileston, 1922) suggested that intravascular hæmolysis did not occur in such patients because of decreased complement. More recently Gardner and Harris (1950) observed a low complement level in three patients with acquired hæmolytic anæmia associated with circulating hæmolysins. Dacie and de Gruchy (1951) observed a low complement level in three of 29 patients with acquired hæmolytic anæmia. Dacie (1954) reported further cases in which there was a low complement level; but apart from noting that cold antibodies were present in all, there was no correlation between a decrease in complement and any clinical or laboratory features. He suggested that the loss of complement might be due to a non-specific change in the plasma proteins.

Van Loghem *et alii* (1952) reported a low serum complement level in cases of paroxysmal cold hæmoglobinuria and a fall to even lower values associated with hæmolysis induced by the Rosenbach test.

In experimental hæmolysis due to incompatible blood transfusion in dogs, Christian *et alii* (1951) found a fall in serum complement level. During a second transfusion two hours later, while the complement level was low, hæmolysis was much slower. The authors suggested that it might have been limited by the decreased complement available.

[1] Received on August 9, 1955. Read at a meeting of The Royal Australasian College of Physicians on May 14, 1955.

In the present investigation serial studies of blood complement have been made in patients suffering from acquired hæmolytic anæmia, and in animals in whom hæmolytic anæmia has been induced by an immune antiserum.

METHODS AND MATERIALS

Experimental Hæmolytic Anæmia

An immune type of hæmolytic anæmia has been induced in guinea-pigs and rats. The antiserum was produced in rabbits by intravenous injections of washed and packed rat or guinea-pig red cells in amounts increasing daily from 0·5 millilitre to 2·0 millilitres (Dameshek *et alii*, 1938). A potent hæmolysin was usually obtained after 10 injections. The rabbit was then bled by cardiac puncture. The serum was stored at 7° C.

Estimations of complement were made in the test animals on several days prior to the intraperitoneal injection of hæmolysin, and then at two, six and ten hours afterwards. They were continued daily or twice daily until the animal died.

Human Acquired Hæmolytic Anæmia

Complement determinations have been made on 14 patients with acquired hæmolytic anæmia giving a positive reaction to the direct Coombs test, and on one other patient apparently suffering from acquired hæmolytic anæmia of immune type, but giving a negative response to the Coombs test. Serial studies were made on 11. Determinations were also made in 10 new-born babies suffering from hæmolytic anæmia of the new-born.

Complement Titration

Complement determinations were made on serum or plasma on the day of collection. In the few cases in which this was not possible the serum was separated and stored at −17° C. A "Veronal" buffer solution containing magnesium chloride was used as the diluent and for the washing of red cells (Mayer *et alii*, 1946). Red cells from the same sheep throughout were collected weekly. The cells, after

being washed, were stored in a refrigerator at 7° C. Preliminary tests had shown that complement estimations with the use of fresh cells and of cells up to seven days old gave identical results.

For each sample of serum a series of eight titration tubes was set up. The dilutions were prepared by making a one in twenty dilution of the serum and adding 0·5 millilitre to tubes containing 1·0, 1·5, 2·0, 2·5 to 4·5 millilitres of diluent. From each tube 0·5 millilitre was transferred to the corresponding titration tube. The latter thus contained increments of serum ranging from 1/120 millilitre to 1/400 millilitre. When it was thought that the complement might be outside this range, a preliminary titration was performed and an appropriate range of serum quantities was set up. Red cell suspension, 0·5 millilitre, was then added to each tube. The suspension was standardized colorimetrically to contain 50,000,000 cells and sensitized with 2·5 M.H.D. of rabbit serum hæmolysin per 0·5 millilitre. After incubation for thirty minutes at 37° C. in a water-bath a further amount of one millilitre of diluent was added and the tubes were centrifuged. The amount of hæmolysis in the supernate was determined in a M.R.C. grey wedge hæmoglobinometer. Complete hæmolysis in the total volume of two millilitres gave a reading of 100%. The quantity of serum giving 50% hæmolysis was determined from a graph relating hæmolysis to the amount of serum in each tube. The results have been expressed as the reciprocal of this value, to give the number of 50% hæmolysis units per millilitre of serum.

To make frequent determinations of complement in the animals, a micro method of complement estimation was developed. This will be reported separately. Briefly, 0·04 millilitre of blood from the ear of a guinea-pig or 0·20 millilitre from the tail of a rat was delivered direct from the opsonic pipette into four millilitres of diluent. The red cells were spun down, and complement was determined on the supernatant, diluted plasma. The complement content of whole blood was thus determined and converted to the plasma value by means of the hæmatocrit measured by the van Allen micro method or calculated from the hæmoglobin value.

RESULTS

Experimental Hæmolytic Anæmia

The effect of a fatal dose of hæmolysin in three guinea-pigs is shown in Figure I. In each case there was a fall of complement to half the preliminary level within twenty-four hours, associated with a fall of hæmoglobin value to 4·8, 3·9 and 2·3 grammes *per centum* respectively. In two animals given equivalent amounts of normal rabbit serum there was a slight fall in hæmoglobin value (to 12·3 and 11·1 grammes *per centum*) and no change in complement.

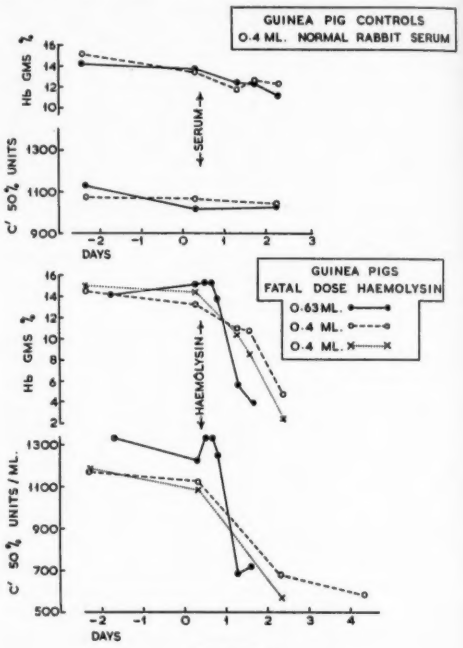

FIGURE I

Complement and hæmoglobin values in three guinea-pigs given hæmolytic serum and in two given normal serum

As serum complement levels in guinea-pigs are approximately four times those in humans,

TABLE I

Normal Values of Serum Complement for Guinea-pigs, Rats and Humans

Subjects	Number Investigated	50% Hæmolysis Units per Millilitre	
		Mean	Range
Guinea-pigs ..	35	1125	935 to 1315
Rats	38	302	168 to 436
Humans ..	77	292	138 to 446

whereas the values in rats are comparable (Table I), more detailed investigations were made on rats. Results are shown in Figure II

and Table II. Immediately after injection of both hæmolytic and normal serum the animals were distressed, breathing rapidly, the hair ruffled and the legs weak and incoordinate. They rested quietly in the cage. These symptoms passed in about one hour. During the subsequent days they lost weight, and as

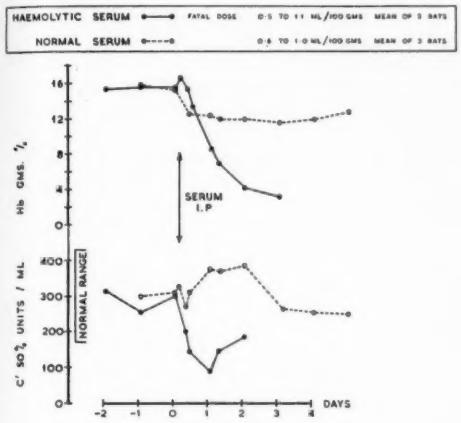

FIGURE II

Mean complement and hæmoglobin values in five rats given hæmolytic serum and in three given normal serum

the anæmia became severe they became progressively weaker. Three animals died after forty-eight hours and the other two died after seventy-two hours. The urine, fæces and all organs examined after death were heavily bile-stained.

All rats given hæmolysin showed a fall in complement level, and this was usually apparent within two hours of injection; the lowest values were recorded after twenty-four hours, being one-sixth to one-third of the preliminary values. After reaching minimum values, the complement level began to rise in spite of continuing hæmolysis. In rat 17 it rose from 18% to 75%, and in rat 18 from 42% to 123% of the original values in twenty-four hours in spite of continuing hæmolysis.

The control rats, given normal rabbit serum in comparable amounts, showed no significant change in complement. There was a fall in hæmoglobin value from a mean of 15·3 grammes *per centum* to 11·6 grammes *per centum* by the third day, but a subsequent rise to 12·9 grammes *per centum* by the fifth day. The cause of this anæmia was not investigated.

Acquired Hæmolytic Anæmia

The mean value of normal serum complement determined in 77 control subjects was 292 units per millilitre with a standard deviation of 77. These subjects comprised 50 hospital patients and 27 normal individuals. There was no significant difference between the two groups.

Repeated observations on control subjects showed that the complement level was relatively constant. Observations on five normal subjects at monthly intervals for nine months showed there was little variation in each subject (Figure III). In one a persistently low complement was found (50 to 70 units per millilitre). The reason for this was not determined. Tests showed that it was not due to a circulating anticomplementary substance.

TABLE II

Complement and Hæmoglobin Values in Three Control Rats given Normal Rabbit Serum and in Five Rats given Rabbit Antiserum to Rat Cells

Dose	Control Experiment						Hæmolytic Anæmia							
	Complement (50% Units per Millilitre)				Hæmoglobin Value (Grammes *per Centum*)		Complement (50% Units per Millilitre)						Hæmoglobin Value (Grammes *per Centum*)	
	Rat 35 (0·6)[1]	Rat 46 (0·6)[1]	Rat 47 (0·6)[1]	Mean	Range	Mean	Rat 18 (0·5)[1]	Rat 17 (0·7)[1]	Rat 31 (0·7)[1]	Rat 32 (0·7)[1]	Rat 19 (1·1)[1]	Mean	Range	Mean
Preliminary ..	365	315	265	315	13·0 to 15·4	15·3	325	310	305	290	270	300	13·4 to 15·2	14·6
After 2 hours ..	365	340	265	323	14·5 to 15·4	14·8	260	310	260	225	260	263	15·6 to 17·2	16·6
After 6 hours ..	340	230	225	265	11·4 to 13·4	12·7	215	240	185	150	230	204	14·2 to 17·2	15·4
After 10 hours ..	405	280	235	306	11·4 to 13·6	12·7	170[2]	160[2]	125	110	140	141	11·2 to 15·3	13·5
After 24 hours ..	335	460	340	378	10·8 to 13·5	12·4	135	55	80	90	75	87	7·4 to 11·0	8·7
After 30 hours ..	415	375	335	375	10·0 to 13·2	12·0	255	135	140	115	80	141	6·0 to 8·0	7·0
After 48 hours ..	445	375	335	385	10·4 to 12·8	11·9	400	230	140	115	60	189	3·2 to 5·0	4·2
After 72 hours ..	265	285	255	268	11·5 to 11·6	11·6	415	215						
After 96 hours ..					11·8 to 12·4	12·0								
After 120 hours ..					12·0 to 13·4	12·9								

[1] Dose in millilitres per 100 grammes.

[2] Interpolated values.

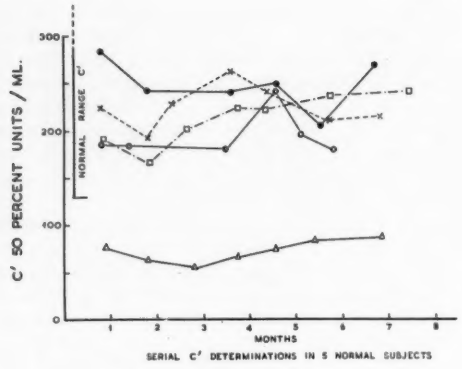

FIGURE III

Complement values in five normal human subjects at monthly intervals

Serial observations in 10 cases of acquired hæmolytic anæmia with positive reactions to the Coombs test and in one with a negative response to the Coombs test are shown in Figures IV to VI. In seven of these 11 patients the complement level was found to be significantly low at some stage of the disease, and in three it was persistently low throughout.

In four other cases one or two measurements were made, but for various reasons it was not possible to continue them. In all these the complement level was within normal limits. A low serum complement level was thus observed in seven of an unselected series of 15 cases of acquired hæmolytic anæmia apparently due to an immune mechanism.

In Table III the complement levels and the duration of their decrease are shown in relation to the clinical and routine laboratory features.

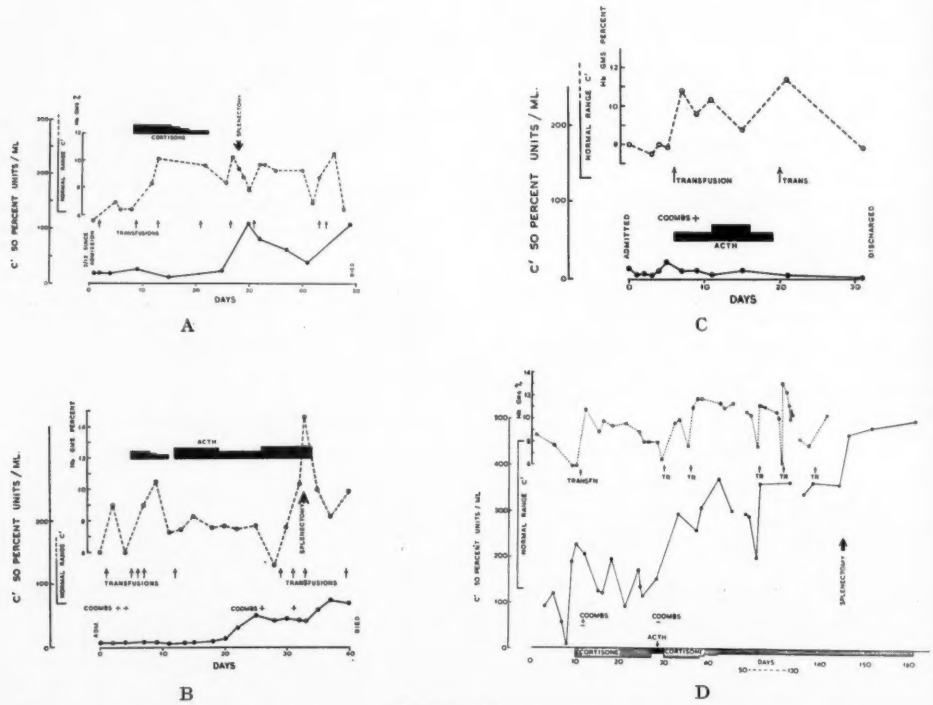

FIGURE IV

Complement and hæmoglobin levels in four patients suffering from chronic acquired hæmolytic anæmia, in whom low complement levels were found. A, patient E.H., female, aged seventy-eight years, suffering from acquired hæmolytic anæmia and pancytopenia. Fatal illness of nine months' duration. Negative response to Coombs test on two occasions. Autopsy revealed marrow aplasia. B, patient C.F., male, aged fifty-six years, suffering from acquired hæmolytic anæmia and Hodgkin's disease. Illness fatal in three months. C, patient S.G., male, aged forty-three years, suffering from acquired hæmolytic anæmia of four years' duration with fatal outcome nine months after investigations. Splenectomy three years previously. Autopsy revealed no primary lesion. D, patient F.L., male, aged thirty-eight years, suffering from acquired hæmolytic anæmia with pancytopenia due to hypersplenism. The primary cause of the latter was not determined, but pancytopenia and hæmolysis were cured by splenectomy

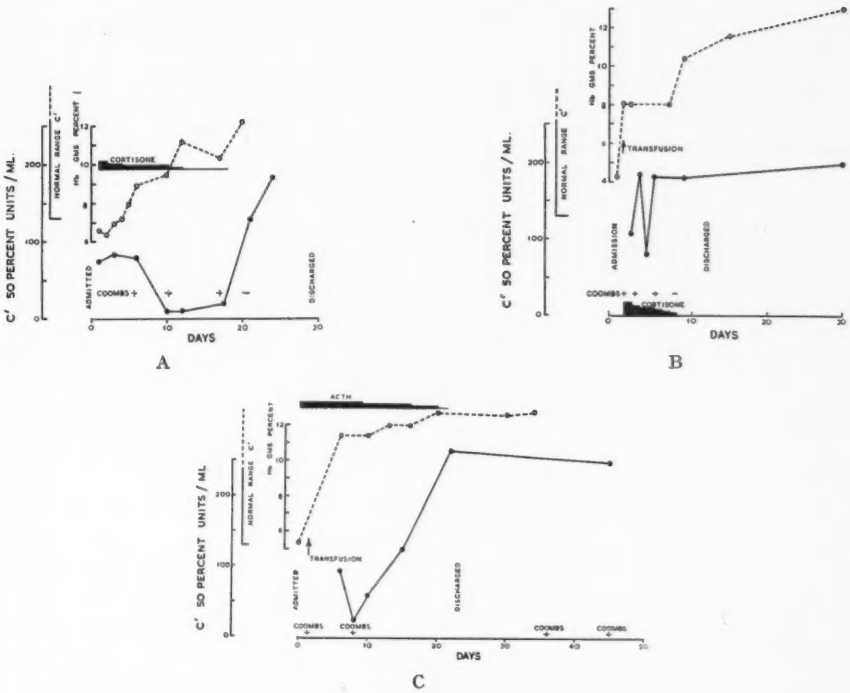

FIGURE V

Complement and hæmoglobin levels in three patients suffering from acute acquired hæmolytic anæmia in whom low complement levels were found. A, patient A.C., female, aged two years, suffering from acquired hæmolytic anæmia, ill for five days before admission to hospital. B, patient M.B., female, aged twenty months, suffering from acquired hæmolytic anæmia, ill for four days before admission to hospital. C, patient H.S., female, aged twenty-one years, suffering from acquired hæmolytic anæmia and diffuse *lupus erythematosus*, ill for seven days before admission to hospital

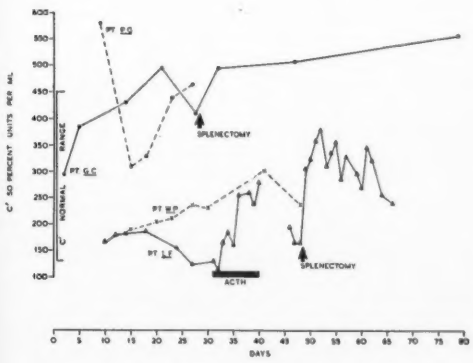

FIGURE VI

Complement levels in four patients with acquired hæmolytic anæmia in whom the complement level remained within the normal range

In 12 babies suffering from hæmolytic disease of the new-born with Rh incompatibility all results were within normal limits (Table IV).

DISCUSSION

The finding of a low blood complement level in some patients with acquired hæmolytic anæmia by the workers referred to above is confirmed by the present results. This fall in level may be due to excessive utilization of complement, or to failure of synthesis, or to both (tests on three subjects showed that it was not due to anti-complementary activity in the serum). The results in the animal experiments in which the complement level was observed to fall within a few hours of injection of hæmolysin suggest that an immune hæmolytic episode is associated with excessive utilization. This may be due to the same mechanism of complement fixation as occurs

TABLE III

Clinical and Routine Laboratory Data for Fifteen Patients Studied

Patient	Age (Years)	Sex	Duration of Illness and Result	Hæmoglobin Value (Grammes *per Centum*)	Reticulocytes (Percentage)	Leucocytes per Cubic Millimetre	Direct Coombs Test Result	Serum Bilirubin Content (Milligrammes *per Centum*)	Primary Diagnosis	Range (Units per Millilitre) and Duration of Low Complement Level
E.H. ..	78	F.	9 months; died	4·7	3	1,750	—	3·5	Marrow aplasia terminally	10 to 109; 49 days
C.F. ..	56	M.	3 months; died	5·5	18·4	16,750	+	2·2	Hodgkin's disease	6 to 75; 44 days
S.G. ..	43	M.	4 years; died	7·5	4	27,500	+	2·0		4 to 25; 32 days
F.L. ..	38	M.	1 year; recovered	5·8	<1	1,750	±	0·4	Hypersplenic syndrome.	3 to 120; 25 days
A.C. ..	2	F.	1 month; recovered	6·3	10	24,000	+	J[1]		10 to 85; (17 days
M.B. ..	20/12	F.	21 days; recovered	4·3	7	34,000	+	J		80 to 105; 5 days
H.S.	21	F.	1 month; recovered	5·5	12	12,250	+	1·5	*Lupus erythematosus*	25 to 100; 8 days
L.F. ..	34	M.	3 months; recovered	6·6	2	4,250	+	1·6		
M.P. ..	52	M.	3 months; recovered	9·0	6	9,500	+		Rheumatoid arthritis	
G.C. ..	3	F.	9 months; recovered	5·4	40	17,000	+	3·2		
P.G.	3	F.	1 month; recovered	5·7	10	19,000	+	0·5		
G.H. ..	3	M.	1 month; recovered	3·1	8	22,000	+	J		
P.M. ..	6	M.	<1 month; recovered	6·6	12	10,000	+			
F.J. ..	56	M.	4 years; recovered	4·6		14,700	+		Chronic lymphatic leuchæmia	
Keith	18	M.	1 month; recovered	5·4	63	4,500	+			

[1] "J," jaundiced.

TABLE IV

Complement Values in Ten Babies Requiring Exchange Transfusion for Hæmolytic Anæmia of the Newborn[1]

Case Number	Sex	Hæmoglobin Value (Grammes *per Centum*)	Serum Bilirubin Content (Milligrammes *per Centum*)	Œdema	Complement (50% Units per Millilitre) (Normal Range 130 to 440)	Result
1	M.	8·0	4·9	—	226	Recovery after persistent anæmia
2	M.	6·3	4·4	+	131	Died at 24 hours
3	F.	9·4	Jaundiced	+	223	Died at 10 days
4	F.	13·2	Jaundiced	+	166	Died at 3 days
5	F.	6·8	5·2	—	223	Recovery after persistent anæmia
6	M.	14·5	3·4	+	150	Died at 3 days
7	M.	16·9	3·5	+	170	Recovered
8	F.	8·8	4·3	—	200	Recovered
9	F.	14·0	1·4	—	228	Recovered
10	F.	12·5	5·1	—	172	Recovered

[1] All mothers had Rh agglutinins during pregnancy, and all the babies' cells gave a positive response to the direct Coombs test. The complement content was estimated on the first sample of blood withdrawn for exchange transfusion.

in immune hæmolysis *in vitro*, thus reflecting lysis (presumably intravascular) of the sensitized cells by complement. The observations of Bonnin and Schwartz (1954) suggest that complement is also necessary for erythrophagocytosis, and thus fixation of complement may also reflect extravascular hæmolysis of the sensitized cells.

That the fall of complement is due to excessive utilization is supported by the finding of a low serum complement level in incompatible transfusion reactions in dogs (Christian *et alii*, 1951), and also as a result of other types of immune reaction in animals (injection of anti-kidney serum in rabbits by Ogawa and Sato, 1938, and injection of albumin and anti-albumin serum in guinea-pigs by Seltzer *et alii*, 1952, confirmed in our own laboratory).

A low complement level has been reported in human serum sickness (Coburn and Pauli, 1939), and the phenomenon is well recognized in acute nephritis (Kellett and Thompson, 1939; Reader, 1948; Fischel *et alii*, 1952), providing further circumstantial evidence that

the low complement level reflects the occurrence of an antigen-antibody reaction.

The second possibility—that a low complement level results from failure of synthesis—does not appear to be the case in one at least of our patients, F.L., whose complement level rose from three to 220 units in twenty-four hours in spite of progressing hæmolysis. In two of the rats, the complement level also rose to normal while hæmolysis continued. A rapid restoration of complement occurs after experimental depletion. Wasserman (1941) demonstrated this by bleeding dogs and returning the cells and heated plasma, and Seltzer *et alii* (1952) by injection of albumin and anti-albumin rabbit serum in guinea-pigs.

Failure of complement regeneration may have been operative in the three patients (C.F., S.G. and E.H.) who died, and whose complement level was persistently low. As the rate of hæmolysis and thus, presumably, complement utilization was not greater than in other chronic cases (patients F.L. and G.C.), in which the complement level remained within normal limits for many months of their illness. The suggested explanation is that complement utilization was excessive in both groups, but regeneration was adequate to maintain a normal level in the latter and inadequate in the former.

It is clear from the present work and from that of other authors quoted that on the one hand the blood complement level falls *in vivo* as a result of an antigen-antibody reaction, and on the other that regeneration of a depleted blood complement content may be very rapid. Therefore in a patient suffering from hæmolysis due to an immune mechanism, the level of blood complement depends on both utilization and regeneration of complement, and to use a metaphor, the level found shows the height of the complement stream, but cannot show either the rate or the direction of its flow. This may be the reason for the lack of correlation between complement levels and other features of such patients noted by Dacie (1954).

In the investigation of a case of acquired hæmolytic anæmia a positive result to the direct Coombs test is usually taken to indicate an immune mechanism in pathogenesis. However, if the Coombs test result is negative, an immune mechanism cannot be excluded, and the pathogenesis of hæmolysis remains in doubt. The present results suggest that a low serum complement level in such cases may be further evidence for an immune mechanism, and in one of the patients this was found in the presence of a negative response to the direct Coombs test.

SUMMARY

A significantly decreased serum complement content was found in seven of 15 patients with acquired hæmolytic anæmia. Serial observations showed that the level of complement remained low throughout the illness in three cases, and in the other four the depression was transitory, lasting from five to twenty-five days.

An immune type of hæmolytic anæmia induced in guinea-pigs and rats was associated with a fall of complement level within a few hours of injection of the antiserum. The fall continued to minimum values, one-sixth to one-third of the preliminary figures, after twenty-four hours.

In some instances, among both humans and animals, the complement, after falling to minimum values, rose to normal within the next twenty-four hours in spite of continuing hæmolysis. This is consistent with reported experimental work showing that complement, depleted acutely, is rapidly regenerated.

It is suggested that the complement level depends on both the rate of utilization by an antigen-antibody reaction, and the rate of regeneration.

In acute cases complement level appears to fall and remains low for some days until regeneration " takes up the slack ". In some chronic cases the persistently low complement level observed may have been due to failure of regeneration.

In diagnosis it is suggested that the finding of a low complement level indicates an immune mechanism of hæmolysis, but a normal level does not exclude such a mechanism.

ACKNOWLEDGEMENT

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TWO ADDITIONAL SEROTYPES OF LEPTOSPIRÆ FROM NORTH QUEENSLAND[1]

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Eleven serotypes of leptospiræ pathogenic to man have been recorded in Australia. All of them were represented in a series of 89 strains isolated in North Queensland during the period from July, 1951, to April, 1953 (Smith *et alii*, 1954). Six leptospiral serotypes new to Australia were isolated in that series. Two of these, *Leptospira canicola* and *L. medanensis*, have previously been described and recorded from other parts of the world. Of the other four, the "Kremastos" and "Szwajizak" strains appear to represent distinct serotypes within the *hebdomadis* serogroup, and the "Robinson" strain a new serotype within the *pyrogenes* serogroup, whilst the "Celledoni" strain has shown no close affinity to any leptospiral serotype with which it has been compared.

The isolation of strains has been continued in North Queensland at the Field Station of the Queensland Institute of Medical Research situated at Innisfail, and now over two hundred have been submitted to the Laboratory of Microbiology and Pathology for typing. Two additional leptospiral serotypes have been found, and are recorded in this paper. One of these is a member of the *grippotyphosa* serogroup and the other is a member of the *australis A* serogroup.

Methods

The general procedure adopted in the isolation and investigation of leptospiral strains has been recorded fully in an earlier paper (Smith *et alii*, 1954). However, in that paper the technique for the agglutinin-absorption tests was not described. For this test the serum to be absorbed is standardized at a titre of 1 : 3000, and one drop of this serum is mixed with 14 drops of a concentrated suspension of leptospiræ. The suspension is prepared by centrifuging 150 millilitres of a well-grown culture in Schüffner's medium in an angle-head centrifuge at 4300 revolutions per minute for one hour and resuspending the sediment in enough normal saline to give 14 drops of heavy suspension. The serum-suspension mixture is placed in a water-bath at 56° C. for two hours and then in a refrigerator at 4° C. for eighteen hours. The absorbed serum is separated by centrifugation and titrated, living cultures being used as antigens.

The "Valbuzzi" Strain

In October, 1954, the "Valbuzzi" strain was isolated from a canecutter at Mossman in North Queensland.

Clinical History of Patient

M.V., a white male, aged twenty-two years, was admitted to the Mossman District Hospital on October 16, 1954, complaining of feverishness and malaise since the previous day. On examination, he appeared ill; his temperature was 103·6° F. and his urine contained albumin. No other significant clinical features were noted. The diagnosis of leptospirosis was considered, but the patient was regarded as suffering from "a pyrexia of unknown origin". Treatment was commenced with penicillin, 500,000 units being given six-hourly. At 10 p.m. on the day of his admission to hospital his temperature reached 106° F., but it fell to normal on the following day. The penicillin dosage was reduced to 100,000 units six-hourly on October 19, and its administration was stopped on October 21. Two days after the patient's admission to hospital the urine was normal, and the patient was discharged from hospital, well, on October 21.

Serological Examination of the Strain

Blood was taken for leptospiral culture on the second day of the patient's illness, and subsequently leptospiræ were isolated and submitted for investigation. Samples of serum were collected on the second, twenty-fifth, twenty-seventh and one hundred and third days for serological examination.

The "Valbuzzi" strain was not agglutinated by immune sera of any of the Australian type strains other than the recently isolated "Esposito" strain (*vide infra*), and then only to 1% of its titre. However, it was agglutinated by a *L. grippotyphosa* (Moscow V) antiserum

[1] Received on July 12, 1955.

to its full titre. An antiserum prepared against the "Valbuzzi" strain agglutinated the Moscow V strain to its full titre, and reacted weakly with two of the Australian type strains.

TABLE I

Agglutination-lysis Reactions of Strain "Valbuzzi"

Serotype[2]	Seroreactions[1]	
	"Valbuzzi" Strain and Type Antisera	Immune Serum of "Valbuzzi" and Type Strains
L. icterohæmorrhagiæ AB (Jackson)	0	0
L. canicola (Berlin)	0	0
L. australis B (Zanoni)	0	0
"Robinson"	0	0
L. australis A (Ballico)	0	1
"Esposito"	1	1
L. pomona (Staines)	0	0
L. medanensis (Ives)	0	0
"Kremastos"	0	0
"Szwajizak"	0	0
L. hyos (Mitis Johnson)	0	0
"Celledoni"	0	0
L. grippotyphosa (Moscow V)[3]	100	100

[1] Titres expressed in percentages of reciprocal for the homologous strain; 0 signifies less than 0·3%.

[2] These are the 11 serologically distinct types previously recorded in Australia and the recently isolated strain "Esposito".

[3] *L. grippotyphosa*, although not known to occur in Australia, was included in our routine screen.

These reactions were to 1% of its titre with both *L. australis A* (Ballico) and the "Esposito" strain. Cross-absorption tests made with immune sera of "Valbuzzi" and Moscow V strains revealed some difference in their antigenic constitution.

The "Valbuzzi" strain is thus the first member of the *grippotyphosa* group of leptospiræ

TABLE II

Cross-absorption Tests: "Valbuzzi" and L. grippotyphosa (Moscow V.)

Immune Serum[2]	Absorbed by Strain	Titre[1] against Strain	
		"Valbuzzi"	Moscow V
"Valbuzzi"	—	100	100
"Valbuzzi"	"Valbuzzi"	0	0
"Valbuzzi"	Moscow V	33	0
Moscow V	—	100	100
Moscow V	"Valbuzzi"	0	3
Moscow V	Moscow V	0	0

[1] Titres expressed in percentages of reciprocal for the homologous strain; 0 signifies less than 1%.

[2] Homologous titres of immune sera standardized at 1 : 3000.

to be isolated in Australia. Its exact relationship to other type strains within this serogroup has yet to be determined. Details of the serological investigations with this culture and the patient's serum are set out in Tables I, II and III.

THE "ESPOSITO" STRAIN

In July, 1954, the "Esposito" strain was isolated from a canecutter at Babinda in North Queensland.

Clinical History of Patient

V.E., a white male, aged twenty-five years, was admitted to the Babinda District Hospital on July 17, 1954. He had first felt ill two days previously and complained of headache, shivers, a "feeling of cold" and a pain in his back, abdomen and both ankles. He had a dry, unproductive cough; his throat was not sore, nor were there any urinary symptoms; his appetite was poor, but he had no nausea or vomiting. He experienced weakness in his legs and photophobia. On examination of the patient, his temperature was 101·8° F., his conjunctivæ were engorged, and large tender glands were present in the axillæ and groin. A few rhonchi were heard at the base of his right lung. There was no neck stiffness, the abdomen was not tender, and the liver and spleen could not be palpated.

TABLE III

Agglutination-lysis Tests with the Sera of Patient from whom the "Valbuzzi" Strain was Isolated

Serotype	Day of Disease			
	2	25	27	103
L. icterohæmorrhagiæ AB (Jackson)	0	0	0	0
L. canicola (Berlin)	0	0	0	0
L. australis B (Zanoni)	0	0	0	0
"Robinson"	0	0	0	0
L. australis A (Ballico)	0	100	100	300
"Esposito"	0	1000	1000	300
L. pomona (Staines)	0	0	0	0
L. medanensis (Ives)	0	0	0	0
"Kremastos"	0	0	0	0
"Szwajizak"	0	0	0	0
L. hyos (Mitis Johnson)	0	0	0	0
"Celledoni"	0	0	0	0
L. grippotyphosa (Moscow V)	0	1000	1000	300
Patient's own culture ("Valbuzzi")	0	3000	1000	300

A diagnosis of leptospirosis was made on clinical grounds, and treatment with penicillin in a dosage of 400,000 units four-hourly was commenced. The temperature fell to normal on July 19. The urine was normal on the patient's admission to hospital, but albuminuria was present on the sixth, seventh and eighth days of the disease. Penicillin therapy was ceased on July 23, and the patient was discharged from hospital, well, on the following day.

Serological Examination of the Strain

Blood was collected for culture on the third day, leptospiræ were isolated, and the strain was submitted for investigation. Serum samples were obtained from the patient on the third and forty-first days of the disease.

The first subculture of the "Esposito" strain did not appear to react with immune sera of any of the Australian type strains, but later subcultures were agglutinated by a *L. australis A* (Ballico) antiserum. The strain

now well established, reacts with the Ballico antiserum to 33% to 100% of its titre, but it is agglutinated to a lesser degree by immune sera of a number of other *L. australis A* strains in our collection. It is agglutinated by an immune serum of the more recently isolated "Valbuzzi" strain to 1% of its titre.

TABLE IV

Agglutination-lysis Reactions of Strains "Esposito" and "Cane-trash"

Serotype	Seroreactions[1]			
	"Esposito" Strain and Type Antisera	Immune Serum of "Esposito" and Type Strains	"Cane-trash" Strain and Type Antisera	Immune Serum of "Cane-trash" and Type Strains
L. icterohæmorrhagiæ AB (Jackson)	0	0	0	0
L. canicola (Berlin)	0	0	0	0
L. australis B (Zanoni)	0	0	0	0
"Robinson"	0	0	0	0
L. australis A (Ballico)	33 to 100	10	100	10
L. pomona (Staines)	0	0	0	0
"Valbuzzi"	1	1	1	0
L. medanensis (Ives)	0	0	0	0
"Kremastos"	0	0	0	0
"Szwajizak"	0	0	0	0
L. hyos (Mitis Johnson)	0	0	0	0
"Celledoni"	0	0	0	0

[1] Titres expressed in percentages of reciprocal for the homologous strain; 0 signifies less than 0·3%.

An antiserum prepared against the "Esposito" strain agglutinated the Ballico strain only to 10% of its titre. It has exhibited similar reactions to 3% to 10% of its titre with

TABLE V

Cross-absorption Tests: "Esposito" and L. australis A (Ballico)

Immune Serum[2]	Absorbed by Strain	Titre[1] against Strain	
		"Esposito"	Ballico
"Esposito"	—	100	10
"Esposito"	"Esposito"	0	0
"Esposito"	Ballico	33	0
Ballico	—	33 to 100	100
Ballico	"Esposito"	0	100
Ballico	Ballico	0	0

[1] Titres expressed in percentages of reciprocal for the homologous strain; 0 signifies less than 1%.

[2] Homologous titres of immune sera standardized at 1 : 3000.

all recently isolated *L. australis A* strains. It does not react with any other Australian serotypes except "Valbuzzi", and then only to 1% of its titre. Cross-absorption tests performed with Ballico and "Esposito" antisera and also with the patient's convalescent serum have indicated a pronounced difference in the antigenic structure of the two strains.

THE "CANE-TRASH" STRAIN

Another strain of leptospira was isolated from cane trash on the cane farm where our patient, from whom the "Esposito" strain was isolated, was working prior to his illness.

TABLE VI

Cross-absorption Tests: "Cane-trash" and L. australis A (Ballico)

Immune Serum[2]	Absorbed by Strain	Titre[1] against Strain	
		"Cane-trash"	Ballico
"Cane-trash"	—	100	10
"Cane-trash"	"Cane-trash"	0	0
"Cane-trash"	Ballico	33	0
Ballico	—	100	100
Ballico	"Cane-trash"	0	100
Ballico	Ballico	0	0

[1] Titres expressed in percentages of reciprocal for the homologous strain; 0 signifies less than 1%.

[2] Homologous titres of immune sera standardized at 1 : 3000.

The details of the isolation of this "Cane-trash" strain will be reported elsewhere by others. The "Cane-trash" strain was found by cross-absorption tests to differ antigenicaly

TABLE VII

Cross-absorption Tests: "Esposito" and "Cane-trash".

Immune Serum[2]	Absorbed by Strain	Titre[1] against Strain	
		"Esposito"	"Cane-trash"
"Esposito"	—	100	100
"Esposito"	"Cane-trash"	0	0
"Cane-trash"	—	100	100
"Cane-trash"	"Esposito"	0	0

[1] Titres expressed in percentages of reciprocal for the homologous strain; 0 signifies less than 1%.

[2] Homologous titres of immune sera standardized at 1 : 3000.

from *L. australis A* (Ballico), but to be homologous with the "Esposito" strain. Details of the serological investigations of the "Esposito" and "Cane-trash" strains and of the patients' serum are set out in Tables IV, V, VI, VII, VIII and IX.

TABLE VIII

Agglutination-lysis Tests with the Sera of Patient from whom the "Esposito" Strain was Isolated

Serotype	Day of Disease	
	3	41
L.icterohæmorrhagiæ AB(Jackson)	10	300
L. canicola (Berlin)	0	0
L. australis B (Zanoni)	0	0
"Robinson"	0	0
L. australis A (Ballico)	0	300
L. pomona (Staines)	0	0
L. medanensis (Ives)	0	0
"Kremastos"	0	0
"Szwajizak"	0	0
L. hyos (Mitis Johnson)	0	0
"Celledoni"	0	0
L. grippotyphosa (Moscow V) ..	0	0
Patient's own culture ("Esposito")	0	1000

TABLE IX

Absorption Tests with the Serum of Patient from whom the "Esposito" Strain was Isolated

Serum	Absorbed by Strain	Titre against Strain		
		L. australis A (Ballico)	"Esposito"	*L. icterohæmorrhagiæ* AB (Jackson)
Patient's serum (41st day) ..	—	300	1000	300
Patient's serum (41st day) ..	"Esposito"	0	0	0
Patient's serum (41st day) ..	Ballico	0	300	0

DISCUSSION

The results of our investigation enable us to record the presence in Australia of a member of the *grippotyphosa* serogroup of leptospiræ. At the present time only one strain, "Valbuzzi", has been isolated, but relatively few cultures have been received from the Mossman area of North Queensland where this strain was obtained. Its antigenic structure, as shown by agglutinin-absorption tests, appears to differ slightly from that of *L. grippotyphosa* (Moscow V), but whether or not it is to be regarded as a distinct serotype is dependent upon the results of further investigation.

The two strains "Esposito" and "Cane-trash" possibly represent a serotype related to, but distinct from, *L. australis A* (Ballico). Wolff (1953) has expressed the opinion that "two strains are considered to be heterologous if the antiserum of each strain after absorption by the other strain retains at least 10 per cent. of its original titre when retested against the homologous strain". Since these criteria are fulfilled in the absorption tests of the Ballico and "Esposito" strains, we have provisionally accepted the "Esposito" strain as a distinct serotype, and incorporated it in the routine diagnostic tests in use in this laboratory.

SUMMARY

Two type strains of leptospiræ pathogenic to man and hitherto unreported in Australia are recorded from North Queensland.

One of these, the "Valbuzzi" strain, is a member of the *grippotyphosa* serogroup of leptospiræ; the other, the "Esposito" strain, appears to show sufficient antigenic variation from *L. australis A* (Ballico) to be regarded as a separate serotype within the *australis A* serogroup.

A strain of leptospira recovered from cane trash on a sugar cane farm was found to be homologous with the "Esposito" strain.

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EXPERIMENTS ON THE HEPATO-RENAL SYNDROME[1]

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IN certain conditions associated with jaundice, necrosis of both liver cells and renal epithelium is found—the so-called hepato-renal syndrome. In this syndrome there is selective necrosis of the proximal convoluted tubules of the kidney, the damaged cells being bile-stained, whilst the collecting tubules contain pigment casts. The liver shows the diverse pathological changes of the processes responsible for the high plasma bilirubin level, while the circulating bile may aggravate the liver-cell necrosis, especially in moribund cells (Dible, 1951).

There have been two main views concerning the ætiology of the syndrome. It is thought either that there is derangement of liver function such as can follow some major surgical procedure, with subsequent renal damage, or that the kidney is independently affected by severe toxæmia. In either case the tubular necrosis is thought to be caused by toxins which are released by, or not detoxicated by, a diseased liver. There are three factors which are commonly associated with renal necrosis, namely, an elevated serum bilirubin level, necrosis of liver parenchyma and shock.

With these factors in view, the hepato-renal syndrome was investigated from several angles.

The relationship of hyperbilirubinæmia to renal tubular necrosis was first investigated by producing obstructive jaundice in experimental animals. The effects on the kidneys of other pigments have been reported previously. Corcoran and Page (1945), working with rats, produced renal ischæmia with hind-limb tourniquets and injected an extract of beef myoglobin intravenously at the same time. This produced a clinical and histological picture resembling the crush syndrome in the human. Their controls were practically normal.

A reproducible, recoverable hæmoglobinuric "nephrosis" has been produced in dogs by Rosoff and Walter (1952), by combining renal anoxia, produced either by shock or as a result of unilateral renal artery occlusion, with intravenous infusions of oxyhæmoglobin or methæmoglobin. The lesion produced was characterized by swelling and exfoliation of the epithelial cells mainly of the proximal convolution. Their controls that received the pigment alone were normal or showed minimal changes. It seemed feasible that bilirubin might have similar toxic properties under certain circumstances.

In the second series of experiments relating to the influence of hepatic necrosis, damage was induced by subcutaneous injections of carbon tetrachloride.

In a review of the ætiology of tubular necrosis in warfare, Maegraith *et alii* (1945) concluded that shock was an invariable factor, though the primary cause differed widely in various cases. It seemed advisable, therefore, to investigate the role which shock played in combination with the other two factors in the hepato-renal syndrome.

Two series of experiments were thus designed. In the first the effects of jaundice and shock were investigated separately and then together. In the second experiment the relationship of hepatic necrosis, jaundice and shock was investigated in various combinations.

METHOD

Albino rats of either sex and weighing between 150 and 250 grammes were used.

Obstructive jaundice was produced by division of the common bile duct between ligatures under ether anæsthesia. This division was made above the entrance of the highest of the pancreatic ducts, for when the bile duct was tied lower down, fat necrosis of the pancreas developed.

Carbon tetrachloride was used to produce hepatic necrosis. This substance is known to cause a centrilobular zonal necrosis of the liver, but in large doses will also produce tubular necrosis. A dosage was established which would produce the former, but rarely the latter. This proved to be 0·2 millilitre per hundred

[1] Received on August 29, 1955.

[2] Research Fellow in Pathology, University of Sydney, 1954.

grammes of body weight in a single subcutaneous injection ; it produced the desired lesion in the liver within twenty-four hours, which was maximal on the second day and healed within a week.

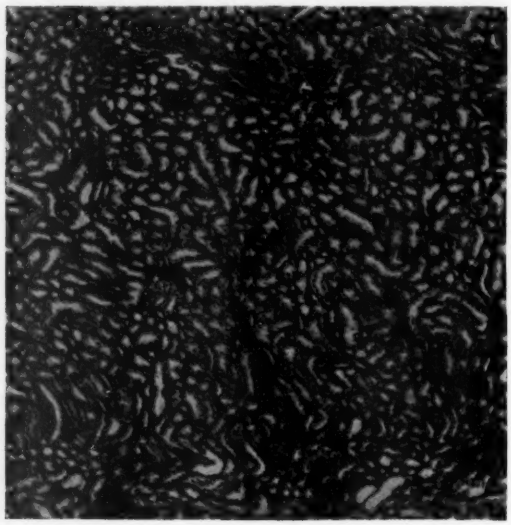

FIGURE I

Normal rat's kidney. The cortex is seen in the upper part of the picture, the medulla in the lower part. The wide band of the boundary zone is in between. (Hæmatoxylin and eosin stain, ×75)

Shock was produced by the application for four and a half hours of a tight tourniquet to one hind limb from ankle to hip. This produced considerable swelling of the limb and shock rapidly developed.

RESULTS

Experiment I : The Effects of Jaundice and Shock

Fifteen animals ((*a*), Table I) were subjected to shock alone. All animals survived in apparently normal health. They were sacrificed between the third and fifth days. Two showed minimal tubular lesions. The remaining 13 had normal kidneys.

Thirty rats were divided into two groups of 15 and the bile ducts of all were divided. By the third day the rats had become deeply jaundiced, but otherwise seemed to be in normal health. At this stage one group of 15 ((*c*), Table I) was subjected to shock by the application of a tourniquet, which was removed four and a half hours later. The limb became greatly swollen and the animals deeply shocked. Three animals died at this stage. The remaining 12 recovered after a few hours and were killed during the next week at varying periods or as they became moribund.

TABLE I

Experiment I : The Effect of Jaundice and Tourniquet Shock on the Rat Kidney

Group	Bile Duct Tied	Tourniquet Applied	No Lesion	Renal Lesion	
				Slight	Severe
(*a*)	—	15	13	2	—
(*b*)	14 survived	—	13	1	—
	1 died	—	—	—	1
(*c*)	12 survived		—	—	12
	3 died		—	3	—

The 15 controls ((*b*), Table I), which had not had a tourniquet applied, were killed at the same intervals, with the exception of one which died of peritonitis.

Macroscopic examination showed little difference between the two groups. The kidneys were swollen and bile-stained with a pale band in the boundary zone between cortex and medulla.

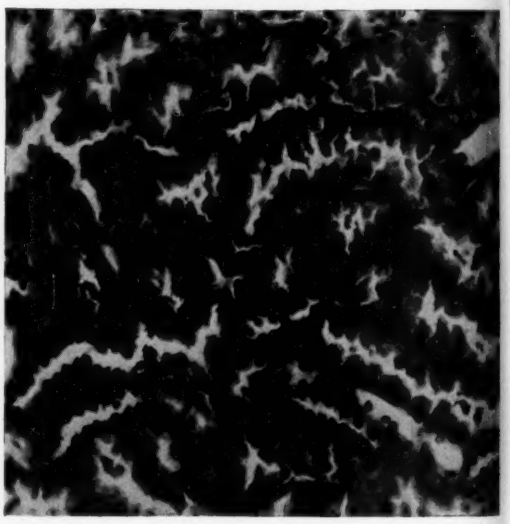

FIGURE II

Proximal tubules with an occasional distal tubule in a normal rat's kidney in the boundary zone (Hæmatoxylin and eosin stain, ×300)

The livers were enlarged, and in many cases there were large green infarcts which may have been caused by the balloon-like distension of the common bile duct pressing on the portal vein.

Microscopic examination showed a great difference between the two groups. The twelve rats which survived the shock of a tourniquet and the one control which died of peritonitis had similar renal changes. The part affected in each case was the pale band between the cortex and medulla (see Figure III). The part of the nephron affected was the lower portion of the proximal convolution in every case, and every tubule seemed to be affected. The epithelial cells were vacuolated and greatly swollen. The cytoplasm had become aggregated into clumps of granules, and although nuclei had disintegrated here and there, most of them were normal but had moved toward the lumen. In places the swollen cells had become desquamated, an empty tubule being left. In most places the large vacuolated epithelial cells had occluded the lumen with only a little eosinophilic material in the middle (see Figure IV). Many cell boundaries were disrupted, but the basement membranes were intact, and although all the tubules were severely damaged there was little actual necrosis. The distal part of the tubule was dilated and contained an occasional cast. There was no interstitial reaction and the glomeruli were unaffected.

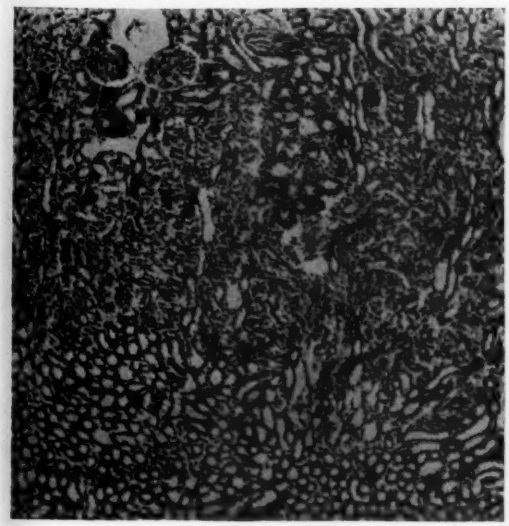

FIGURE III

Degenerate proximal tubules in the boundary zone of a rat rendered jaundiced and later subjected to shock with a tourniquet. (Hæmatoxylin and eosin stain, ×75)

Of the remaining 14 controls and the three animals that died of shock in the early stages,

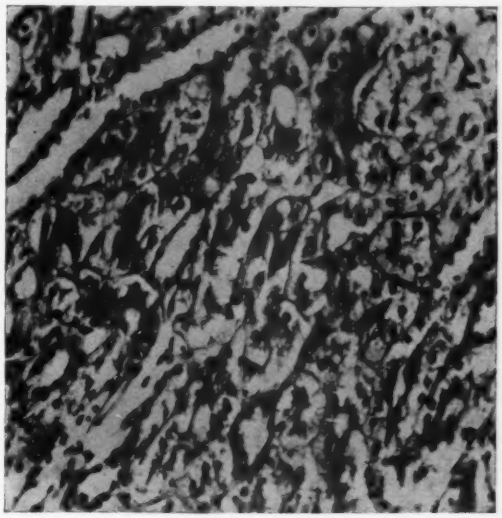

FIGURE IV

Higher magnification of Figure III. The cells are vacuolated, their membranes ruptured and there is a diminished number of nuclei. The distal tubules are normal. (Hæmatoxylin and eosin stain, ×300)

13 had histologically normal kidneys, but in the other four there was slight swelling of the cells of the lower part of the proximal convolution with no other abnormality. In the case of the three animals that died there probably was not enough time for pronounced histological changes to occur.

Experiment II: The Effects of Hepatic Necrosis, Jaundice and Shock

The results of Experiment II are set out in Table II.

Of 10 control rats that had received carbon tetrachloride, only two showed mild vacuolation of the proximal convoluted tubules of the kidneys.

Three groups of animals were used to examine the effects of the following combinations: (*a*) Obstructive jaundice with hepatic necrosis; (*b*) hepatic necrosis with tourniquet shock; (*c*) a combination of all three.

(*a*) Eight animals had their common bile ducts divided and on the third day received

carbon tetrachloride. They were then sacrificed three to four days later. Examination of the livers showed rapid regeneration of cells. The kidneys were affected in seven cases, and normal in the remaining one. Of the seven, one showed severe hydropic changes with granulation of cytoplasm and desquamation of the epithelium. The other six had only mildly swollen tubules but no other cellular derangement. As in the previous experiment the lesions were confined to the proximal tubules in the boundary zone.

(*b*) Previous workers have found that renal damage can be produced when shock is combined with a dose of a nephrotoxin which would be too small to produce any damage if used alone. Therefore a group of eight rats was given the same dose of carbon tetrachloride, followed two days later by the application of a tourniquet.

TABLE II

Experiment II: The Effect of Hepatic Necrosis, Jaundice and Tourniquet Shock on the Rat Kidney

Group	Carbon Tetrachloride	Bile Duct Tied	Tourniquet Shock	No Lesion	Lesion	
					Slight	Severe
Control	10	—	—	8	2	—
(*a*)	8	8	—	1	6	1
(*b*)	8	—	8	5	3	—
(*c*)	12	12	12	1	—	11

There was no obvious jaundice. They were then killed from the third to the fifth day. Five had normal kidneys, but the other three had moderately vacuolated proximal tubules in the boundary zone without any necrosis.

(*c*) To examine the combined effects of a raised plasma bilirubin level, hepatic necrosis and shock, 12 rats were rendered jaundiced as above. Three days later they were given an injection of carbon tetrachloride and two days later a tourniquet was applied. They were then killed, either as they became moribund or as they reached the fourth day. They all became severely ill. Of the 12 rats the kidneys of one were normal, and in the other 11 the kidneys were moderately to severely damaged, the lesions being identical with those in the animals which were rendered jaundiced and then subjected to shock in the first experiment.

DISCUSSION

The rationale of using the tourniquet to induce shock is based on the work of Corcoran and Page (1945). Using dogs, they showed that a tourniquet applied to a hind limb will reduce the renal blood flow by as much as 80% in the first two hours even while the systemic blood pressure is maintained. Later when the tourniquet is released the blood pressure falls, with increase in the shock. Although a tourniquet will produce a picture comparable to the crush syndrome in animals like the cat, whose muscles contain myoglobin, in the rat, which has very little myoglobin, structural changes are confined to a little cloudy swelling in an occasional tubule.

It seems that the ætiology of acute tubular necrosis, heterogeneous as it is, is based on three major causes: nephrotoxins, pigments and ischæmia. These are frequently combined and often one may be the cause of the other.

In the hepato-renal syndrome the pigment bilirubin is often associated with some form of trauma leading to ischæmia resulting from shock.

In the foregoing experiments a high concentration of bilirubin led to mild tubular changes in three of the 14 controls which were jaundiced. When shock and presumably a severe degree of renal ischæmia were superadded, all 12 animals developed extensive tubular damage.

A theory has been advanced by Hampton and Mayerson (1950) that the changes in the kidney due to blood pigments may be due to the iron in the hæme molecule, which may take the form of ferric hydroxide and possibly interfere with the cytochrome system of the tubular cells. This theory is, of course, untenable with respect to bilirubin.

The role of hepatic necrosis was investigated in dogs by State *et alii* (1950), who injected carbon tetrachloride directly into the portal vein. This caused severe hepatic necrosis, and in animals which survived six hours there was hydropic degeneration of the proximal convoluted tubule in the kidney. This they ascribed to the shock produced by a large volume of blood becoming trapped in the portal circulation. But as such large doses of carbon tetrachloride would produce changes in the kidney, their findings are inconclusive.

In the second experiment (II (*a*)) the combination of a high plasma bilirubin level and temporary hepatic necrosis produced renal lesions which were similar to those produced by the former alone. When the animals were also subjected to shock, the lesions were similar to those produced by bilirubin and shock alone. The occasional tubular changes found when carbon tetrachloride and shock were used together was more likely to be due to the

combined effect of the two on the renal tubules directly, than to any hepatic necrosis. Whether the degree of hepatic failure induced by the poison in any way resembles that in human hepato-renal failure is debatable. It is certain that in this experiment hepatic necrosis seems to play a very small part.

The selective damage produced by bilirubin agrees with the observations of Oliver *et alii* (1951). By microdissection of tubules damaged by various means they classified tubular lesions into two groups. There is firstly the nephrotoxic lesion caused by a poison which is circulating in the blood-stream and is distributed evenly to every nephron in the kidney. The effect is upon the same portion of the proximal convoluted tubule, the epithelium of which is shed. The basement membrane and the rest of the nephron escape.

Secondly there is the lesion which they call tubulorrhexis. It has a random distribution in the kidney and not all the nephrons are affected, nor is the lesion an invariable one. The damage may be localized at any point from the origin of the tubule to the collecting distal end. One of the most frequent sites is the second or lower half of the proximal convolution at the boundary zone. In this type of lesion the basement membrane is also disrupted, which leads to permanent damage, whereas the first group was recoverable.

This random distribution can be explained by a reduced renal blood flow, which under experimental conditions has frequently been shown to be patchy, some areas being congested and others bloodless (Schlegel and Moses, 1950).

Bilirubin seems to act as a nephro-toxin. It has a very diffuse distribution in the kidney and affects every tubule equally. From these experiments it appears to be a predisposing factor and to exert its full toxicity on the kidney only when a precipitating factor such as shock is also present.

SUMMARY

Experiments have been carried out on rats to determine the role of jaundice, hepatic necrosis and shock in the renal lesion of the hepato-renal syndrome.

Jaundice alone produced a slight tubular lesion in two of 15 animals. Jaundice with shock produced severe necrosis in all animals.

Hepatic necrosis produced by carbon tetrachloride appeared to play little part in the renal lesion, which depended on the degree of shock and of bilirubinæmia.

ACKNOWLEDGEMENTS

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ACUTE DISSEMINATED LUPUS ERYTHEMATOSUS IN A CHILD[1]

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ACUTE disseminated *lupus erythematosus* has been included in the general group of collagen diseases by Klemperer *et alii* (1942), and is usually regarded as a disease which occurs in females during the reproductive span of life.

The case to be presented is of considerable interest because of the occurrence of the disease in a girl, aged eight years, because of changes in the serum proteins following treatment with ACTH and cortisone, and because there was radiographic evidence of pulmonary infiltration which was considered to be part of the disease in spite of a strong family history of tuberculosis.

CASE REPORT

The patient was a girl, aged eight years, who had developed spastic paralysis of the legs after birth injury, but had been trained to walk. A brother-in-law of the patient's mother had died from acute pulmonary tuberculosis four years prior to the onset of the child's illness, and he had been a frequent companion of the child.

The child first became ill in January, 1954, when she complained of aches and pains in her limbs; but these symptoms were not regarded as serious by her parents, and she was encouraged to be active. In early February she became sunburnt at a picnic, and a rash appeared across her nose, on both cheeks and on her forehead. This rash was still present one month later when she was first examined by a doctor, and although she had no specific symptoms and was afebrile, her parents thought that she was unwell. She was admitted to hospital with the diagnosis of *lupus erythematosus*. Her hæmoglobin value was 10·9 grammes *per centum*, the white cell count was normal, and the erythrocyte sedimentation rate was 16 millimetres in one hour. An X-ray examination of the chest revealed increased mottling in both lungs (Figure I); the Mantoux test failed to produce a reaction (1/1000 old tuberculin), and L.E. cells could not be demonstrated in the patient's peripheral blood.

In early April she contracted gastro-enteritis along with other children in the ward and became febrile (temperature 99° to 100° F.) for the first time. She did not benefit from "Aureomycin", as the pyrexia was more severe, vomiting, diarrhœa and weight loss continued, and the radiological appearances in her chest remained unchanged. At about this time L.E. cells were demonstrated in her peripheral blood, and she was transferred to the Clinical Research Unit, Royal Prince Alfred Hospital, four weeks after the onset of gastro-enteritis.

On her admission to the unit on April 29, she was dehydrated, apathetic and under weight and had mild pyrexia (temperature rising to 100° F.). Physical examination revealed no abnormality other than a salmon pink maculo-papular rash scattered on her cheeks, nose, forehead and neck. Her urine contained acetone, and two to 13 leucocytes and some clumps per high-power field, but was free from albumin and red cells.

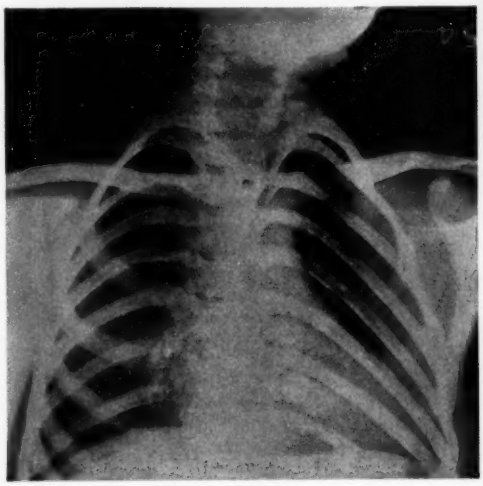


FIGURE I

X-ray film of the chest, showing increased mottling in both lungs

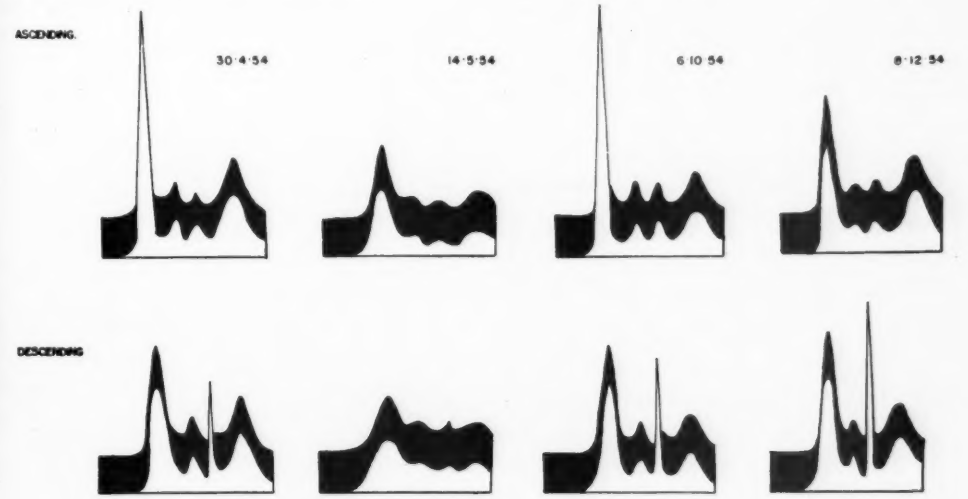
The following results were obtained on laboratory investigation: The blood urea content was 24 milligrammes per 100 millilitres, the serum chloride content 114 milliequivalents per litre, the serum sodium content was 150 milliequivalents per litre, the serum potassium content 5·7 milliequivalents per litre, and the serum bicarbonate content 20 milliequivalents per litre. The serum contained no agglutinins against *Salmonella typhi* and *paratyphi*, *Proteus vulgaris* or *Brucella abortus*, and the results of Paul-Bunnell, Kline and Coombs tests were negative. The hæmoglobin value was 10·0 grammes *per centum*, the hæmatocrit was 39% and the white cell count was normal (the total white cells were 7200 per cubic millimetre, 73% being neutrophile cells, 21% lymphocytes, 5% monocytes and 1% eosinophile cells); the platelet count was 206,000 per cubic millimetre.

[1] Received on May 12, 1955.

[2] Adolf Basser Fellow in Clinical Research.

The erythrocyte sedimentation rate was 38 millimetres in one hour (Wintrobe) (normal, up to 10 millimetres in one hour). The zinc sulphate turbidity of her serum was 37 units (normal, up to 15 units), the total serum protein content was 7·7 grammes *per centum*, and electrophoresis showed low albumin, raised α-2 globulin and much raised γ globulin concentrations (Figure II).

On May 2, when she was rehydrated, the depot administration of ACTH ("Androcort"), 20 units per day by intramuscular injection, was commenced.



	%	g%	%	g%	%	g%	%	g%
ALB.	42	3·2	48	2·5	47	3·8	40	3·3
GLOB.	58	4·5	52	2·7	53	4·2	60	4·9
α_2	13	1·0	15	0·8	13	1·0	10	0·8
β	12	0·9	10	0·5	9	0·7	13	1·1
γ	33	2·6	27	1·4	31	2·5	37	3·0
%	0·77%		0·82%		0·89%		0·67%	

FIGURE II

Tiselius electrophoresis patterns, showing variations in the serum protein fractions during the child's illness

Considerable improvement occurred in the next two days, so that she ate and drank and was less apathetic. An X-ray film of her chest was reported as showing no abnormality; but, in view of the previous mottling and a family history of tuberculosis, streptomycin was given in a dose of 0·5 gramme twice a week, and isonicotinic hydrazide in a dose of 50 milligrammes daily.

Improvement was maintained, so that ten days after ACTH treatment was begun the rash had almost faded and she was afebrile; she was now given cortisone acetate, 50 milligrammes twice daily by mouth, together with 55 milliequivalents of potassium per day.

The results of investigations carried out after two weeks were as follows. The Mantoux test failed to produce a reaction (1/10 strength old tuberculin), and the erythrocyte sedimentation rate was 16 millimetres in one hour. The serum chloride content was 90 milliequivalents per litre, the serum sodium content 130 milliequivalents per litre, the serum potassium content 5·5 milliequivalents per litre, the serum bicarbonate content 32 milliequivalents per litre, the serum zinc sulphate turbidity 15·5 units, the total serum protein content 5·2 grammes *per centum*, and the electrophoretic findings in the serum were as tabulated (Figure II).

Antituberculosis agents were suspended on May 18, when she was allowed to become progressively more ambulant, and she continued to receive 100 milligrammes of cortisone daily, but did not develop œdema or hypertension. One month after her admission to hospital L.E. cells were still demonstrable in her peripheral blood, the erythrocyte sedimentation rate was nine millimetres in one hour and the hæmoglobin value was 10·5 grammes *per centum*. The daily dose of cortisone was decreased to 50 milligrammes six weeks after her admission to hospital, and a slight "rebound" phenomenon occurred; she became anorexic, apathetic and febrile for twenty-four hours. An X-ray examination of her lungs still revealed no abnormality, but L.E. cells were still demonstrated in her peripheral blood.

She was discharged from hospital on June 24, and has been carefully followed for eleven months. For the first six months she was taking 50 milligrammes of cortisone daily; but as she commenced to have evening rise of temperature and appeared to be regressing, the dosage of cortisone was increased to 75 milligrammes daily, with immediate clinical improvement; she has had daily potassium supplements and has shown no overt œdema or hypertension. The anæmia was completely corrected by mid-July, when the hæmoglobin value was 15·3 grammes *per centum*; but in November it fell to 13·0 grammes *per centum*. L.E. cells have been demonstrated in the peripheral blood on all occasions when they have been sought. Electrophoresis of her serum proteins carried out in October and in December showed that the γ globulins were still increased; the results are set out in Table I.

ELECTROPHORESIS

Electrophoresis was carried out by the classical Tiselius method in a Perkin-Elmer apparatus incorporating a Philpot-Svenson optical system and Longworth's scanning method. Serum was diluted 1:4 with a "Veronal" saline buffer at pH 8·6 and ionic strength 0·1, against which it was dialysed in the cold (4° C.) for eighteen hours. Protein concentrations were determined by the method of measuring the areas beneath the pattern with a planimeter utilizing minimum ordinates.

DISCUSSION

The diagnosis of acute disseminated *lupus erythematosus* may at times be difficult to substantiate; but in this case it is well established. The patient had a characteristic rash in a butterfly distribution on her face after over-exposure to sunlight, L.E. cells as described by Hargraves *et alii* (1948) were demonstrated on numerous occasions in her peripheral blood, and hyperglobulinæmia was present prior to treatment. Gold and Gowing (1953) consider the diagnosis of acute disseminated *lupus erythematosus* to be in doubt if the last-mentioned finding is absent.

Baehr and Pollack (quoted by Ehrick, 1952) reported that 95% of cases occur in females aged between twenty and forty years; although many large series of cases of acute disseminated *lupus erythematosus* have been reported, only seven patients in the first decade have been found, and in some of these series neither age nor sex was stated. Tumulty and Harvey (1949) mention one patient in the first decade, and Shearn and Pirofsky (1952) mention two such patients; the latter authors also describe a third patient in whom the disease began at the age of three years, when it was diagnosed as rheumatic fever. The condition has been reported in two girls aged seven years (one case by Baehr *et alii*, 1935, and one by Denzer and Blumenthal, 1937), and the latter authors also quote a report by Madden (1932) of the disease in a child, aged five years. Dubois *et alii* (1952) encountered the disease in a Mexican girl, aged eight years, in whom it presented as acute hæmolytic anæmia.

The presence of abnormal globulins is partly responsible for the increased erythrocyte sedimentation rate, and may be responsible for occasional false positive Wassermann and Kahn reactions. Multiple antibodies may form after blood transfusion (Callender *et alii*, 1945), and the Coombs test may also produce a positive result, as in eight of 26 cases described by Gold and Gowing (1953). The presence of an abnormal γ globulin which differs immunologically from normal γ globulin (Haserick and Lewis, 1950) has been shown in acute disseminated *lupus erythematosus*. It is thought to be transmitted across the placenta (Bridge and Foley, 1954), and to be associated with the transient appearance of L.E. cells in the blood of the new-born child.

It has been suggested in an annotation in *The Lancet* (1954) that acute disseminated *lupus erythematosus* may belong to the group of "dysgammaglobulinæmias", a term suggested by Ehrick (1952). Ehrick and Haserick (1951) stated that the L.E. cell might be absent if the serum globulin concentration was low; but our patient's serum γ globulin level was rapidly reduced to normal and the L.E. phenomenon persisted. L.E. cells are absent in many cases of acute disseminated *lupus erythematosus* (Gold and Gowing, 1953; Zimmer and Hargraves, 1952) and Dubois *et alii* (1952) state that in as many as 20% the cells may not be found.

Reiner (1950), in a study of five cases of acute disseminated *lupus erythematosus*, found a protein pattern which had not been demonstrated previously, although 800 sera from patients with a variety of other conditions had been tested. In the five sera studied the total protein content was within normal limits, but the albumin content was decreased and all the globulin fractions were raised; in particular the α-2 globulin content was increased to twice the normal amount and did not usually return to normal either during treatment with cortisone or during a spontaneous remission.

The buffer used to separate the present patient's serum protein constituents by electrophoresis does not permit the separation of α-1 globulin, which migrates with the albumin fraction. However after twelve days' treatment with ACTH, the total proteins fell by 2·5 grammes *per centum*, the γ globulins fell by 1·2 grammes *per centum*, the albumin fell by

0·7 gramme *per centum*, and the α-2 and β components fell by small amounts. Cohen and Cadman (1953) investigated five patients with acute disseminated *lupus erythematosus*; they state that "neither the sedimentation rate nor the gamma globulin levels fell early in the course of treatment, but after several months they tended to approach normal levels". There is no obvious relationship between the γ globulin level in the untreated patient and the duration of the disease (Soffer and Bader, 1952).

The response to ACTH and cortisone was encouraging, as the fever diminished and the clinical state improved. The face rash subsided in ten days, but a violaceous hue in the infra-orbital region persisted. The child was not given a large dose of cortisone, as the Cushing-like state recommended by some authorities (Dubois *et alii*, 1952) was not desired; even so, her face is rounded and "moon"-like, but overt œdema has not occurred.

The X-ray evidence of pulmonary infiltrations was disturbing in view of the child's close contact with a sufferer from active tuberculosis. The pathogenesis of acute disseminated *lupus erythematosus* and that of pulmonary tuberculosis appear to be unrelated, although cases are reported in which either these diseases coexisted (Tumulty and Harvey, 1949), or the administration of ACTH or cortisone activated a latent tuberculosis focus (Walker, 1952; Harris-Jones and Pein, 1952). A survey of the literature of acute disseminated *lupus erythematosus* by Rapaport *et alii* (1953) showed that, although pulmonary lesions are common, they are frequently atypical and difficult to evaluate; these authors suggest that the lungs may be as common a site of initial involvement as are the heart, kidneys and serous membranes. Diffuse pulmonary infiltration may be asymptomatic and discovered only by X-ray examination, or severe respiratory symptoms may exist with massive pulmonary consolidation or atelectasis. Rapaport *et alii* suggest that most commonly there are shifting areas of pneumonitis, usually at the lung bases and associated with a pleural reaction; these areas disappear rapidly on treatment with ACTH.

Reviewing the prognosis in acute disseminated *lupus erythematosus*, Cohen and Cadman (1953) stress the difficulty of evaluating therapy when spontaneous remissions are unpredictable. However, they agree that the more acute the systemic disease, the shorter is the life expectancy and the less likely are the remissions. Nevertheless there can be no doubt as to the immediate effectiveness of ACTH and cortisone in systemic disease.

The child has now experienced symptomatic remission for eleven months although L.E. cells are still demonstrable in her peripheral blood; she continues to take 75 milligrammes of cortisone with 55 milliequivalents of potassium daily; she attends school and is progressing well.

SUMMARY

Acute disseminated *lupus erythematosus* occurring in a girl, aged eight years, is reported. ACTH and cortisone therapy rapidly caused the reduction of the hyperglobulinæmia to normal levels. The patient showed radiological evidence of pulmonary infiltrations, which were regarded as unusual manifestations of the disease.

ACKNOWLEDGEMENTS

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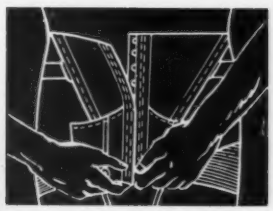

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